ACTA MEDICA

ACADEMIAE SCIENTIARUM HUNGARICAE

TOMUS XIX FASCICULUS 1

AKADÉMIAI KIADÓ, BUDAPEST
1963

ACTA MEDICA

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KIADÓHIVATAL: BUDAPEST V., ALKOTMÁNY UTCA 21

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An die gleiche Anschrift ist auch jede für die Redaktion bestimmte Korrespondenz zu richten.

Abonnementspreis pro Band: 110 Forint. Bestellbar bei dem Buch- und Zeitungs-Außenhandels-Unternehmen »*Kultura*« (Budapest I., Fő utca 32. Bankkonto Nr. 43-790-057-181) oder bei seinen Auslandsvertretungen und Kommissionären.

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TOMUS XIX

INDEX

ELECTROCARDIOGRAPHIC AND CIRCULATORY CHANGES IN PROGRESSIVE MUSCULAR DYSTROPHY

By

F. Solti, E. Zádory and Gy. Bekény

FIRST DEPARTMENT OF MEDICINE (DIRECTOR: PROF. I. RUSZNYÁK) AND DEPARTMENT OF NEUROPATHOLOGY (DIRECTOR: PROF. B. HORÁNYI), UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received October 1, 1961)

In 22 patients suffering from progressive muscular dystrophy on the evidence of clinical examinations and muscle biopsy the following circulatory and ECG changes were revealed.

Stroke volume was frequently diminished and peripheral resistance augmented. The mechanic systole often lagged behind the electric systole.

Electrocardiograms revealed pathological changes in 80 per cent of the cases; these changes were of three types.

The mechanism of electrocardiographic and circulatory changes is discussed.

Introduction

There is an increasing number of observations to show that progressive muscular dystrophy often affects not merely the musculoskeletal system but also the myocardium. The occurrence of an affection of the heart muscles and the resulting cardiac disturbance have been proved by the following observations.

(i) In patients who had died of progressive muscular dystrophy autopsy often revealed similar histological changes in the myocardium and in the skeletal muscles [1, 2, 3, 4].

(ii) In such patients rapidly developing circulatory failure has been observed which had to be ascribed to the disease at issue [5, 6, 7, 8, 9].

In spite of these observations, only a few authors have actually investigated the problem involved and the results are somewhat contradictory. There are hardly any data regarding the systemic circulation in connection with the disease, except concerning pressure in the pulmonary circulation, More numerous are the ECG reports, but their results are also contradictory. The incidence of pathological ECG-changes in connection with progressive muscular dystrophy has not yet been stated, nor has it been determined whether those changes are specific of the disease. Interconnections between ECG changes, circulatory disturbances and the results of muscle biopsy have not yet been elucidated.

The present study deals with electrocardiographic and circulatory changes in progressive muscular dystrophy.

Material and method

The examinations were made in 22 patients suffering from progressive muscular dystrophy, treated at the Department of Neuropathology. Diagnosis was based on clinical evidence and muscle biopsy.

After the usual examinations, the patients were questioned about possible circulatory complaints. In addition to routine circulatory tests the ECG recorded from the extremities and the precordial leads V_1, V_2, V_4 and V_6; 6 chest leads and 3 unipolar extremity leads were examined in half of the cases, too.

Ten patients were subjected to a more detailed circulatory examination, involving determination of the electric systole, the mechanic systole, further the systolic phases and the rate of propagation of the pulse wave, the stroke volume according to WEZLER and BÖGER [10], the cardiac output and the peripheral resistance.

The obserbed changes were then assessed in the light of the patient's complaints, the clinical picture and the muscle biopsy results.

Results

The clinical data are assembled in Table I. Cardiac complaints were mentioned by 8 patients. Symptoms of cardiac decompensation were found in none of the cases. Data regarding circulation are shown in Table II.

The most conspicuous and frequent changes were a decreased stroke volume, and an increased peripheral resistance. Cardiac output was low in most cases. The QT interval was either at or slightly above the highest limit of normal. Compared with the electric systole, the mechanic systole was delayed in 8 out of 10 cases. The second — or, exceptionally, the first — heart sound was split or doubled in cases of disturbed intraventricular conduction. Neither the character nor the degree of the circulatory phenomena revealed any interconnection with the biopsy results.

Table I

Clinical data of patients suffering from progressive muscular dystrophy

No	Initials, sex, age, weight	Clinical appearance and the result of muscle biopsy	Symptom pointing to circulatory disorder	Tension	Pulse rate
1	M. Z., female, 14 years	Ascending form of pseudohypertrophy. Has been ill for 8 years	—	120/80	80
2	Gy. A., female, 17 years	Mild ascending form (2 further cases in the family)	—	120/80	70
3	M. A., female, 18 years	Grave descending form (2 further cases in the family)	Dyspnoea on strain	130/80	70
4	A. B., male, 18 yrs, 38 kg	Ascending form since childhood	—	140/90	70
5	Gy. K., female, 33 years	Ascending form since 15th year of age	Dyspnoea on strain Enlarged liver	120/80	80
6	L. Sz., male, 30 years	Ascending form since 10th year of age	—	125/90	80

No	Initials, sex, age, weight	Clinical appearance and the result of muscle biopsy	Symptom pointing to circulatory disorder	Tension	Pulse rate
7	M. Z., male, 36 years	Ascending form of pseudohypertrophy (2 siblings likewise ill)	Dyspnoea on strain	120/80	88
8	G. M., male, 7 yrs, 20 kg	Ascending pseudohypertrophy during the last 3 years	Palpitation	110/80	90
9	A. A., female, 21 yrs, 46 kg	Descending form during last 2 yrs (a sibling likewise ill)	—	145/100	60
10	M. K., male, 36 years	Grave ascending form since 15th year of age	—	130/80	96
11	J. K., female, 36 yrs, 61 kg	Grave ascending form since 16th year of age	Dyspnoea for the last year. Precordial pain; right heart enlarged	150/100	70
12	J. R., male, 33 yrs, 60 kg	Grave ascending form during the last 4 years	—	130/70	80
13	A. V., female, 39 years	Incipient ascending form since one year (no biopsy)	—	110/75	60
14	K. P., female, 8 years	Mild ascending form during last 5 years	—	110/70	62
15	I. E., male, 14 years	Grave ascending form of pseudohypertrophy since 6th year of age	Palpitation	130/80	90
16	I. K., male, 34 years	Ascending form of pseudohypertrophy during last 10 years	—	130/80	68
17	J. A., female, 19 yrs, 71 kg	Grave ascending form since 14th year of age	—	115/80	76
18	A. K., male, 19 yrs, 78 kg	Grave ascending form since 8th year of age (1 sibling likewise ill)	—	170/90	60
19	P. M., female, 29 years	Ascending form during last 10 years	Dyspnoea on strain. Left heart enlarged	100/70	76
20	L. Gy., male, 31 yrs	Ascending pseudohypertrophy during last 8 years	—	140/80	60
21	Gy. T., male, 31 yrs	Descending form	—	130/80	60
22	E. M., female, 27 yrs	Ascending pseudohypertrophy	Dyspnoea on strain	150/90	78

Table II

Circulatory findings

No	Initials, age, sex Case No.	Blood pressure	Pulse rate	Electric systole E. 1, S_I S_{II}	Mechanic systole	Phonocard.	Pulse wave cm/sec	Stroke volume	Cardiac output litre/min	Peripheral resistance (dyn. cm. sec^{-5})
1	J. K., 36 yrs, female, case 11	150/100	72	330 m.sec 35, 70, 225	350	—	9.20	52.6	3.94	2540
2	J. R., 33 yrs, male case 12	130/70	70	360 m.sec 40, 90, 230	430	—	10.10	38.4	2.69	3130
3	A. V., 39 yrs, fem., case 13	110/75	60	300 m.sec 40, 80, 180	340	—	4.50	50.0	3.00	2400
4	K. P., 8 yrs, fem., case 14	110/70	62	340 m.sec 40, 90, 210	340	2nd sound split	8.00	40.0	2.48	2600
5	I. E., 14 yrs, male case 15	130/80	70	310 m.sec 40, 80, 190	310	—	7.60	40.0	2.80	2200
6	I. K., 34 yrs, male, case 16	130/80	68	340 m.sec 50, 90, 200	380	2nd sound split	7.70	31.0	2.10	4000
7	J. A., 19 yrs, fem., case 17	115/80	76	300 m.sec 40, 80, 180	340	Syst. murmur	6.80	40.0	3.36	2500
8	A. K., 19 yrs, male, case 18	165/100	60	340 m.sec 40, 90, 220	340	—	7.14	62.0	3.70	2830
9	P. M., 29 yrs, fem., case 19	100/70	74	360 m.sec 40, 100, 220	400	2nd sound doubled 1st split	6.56	32.0	2.40	2700
10	L. Gy., 31 yrs, male, case 20	140/80	60	400 m.sec 50, 110, 240	440	2nd sound split	8.90	60.0	3.60	2100

Table III shows the electrocardiographic changes. They were pathologic in 10 cases and belonged to three types.

Table III

Survey of electrocardiographic changes

Type of ECG tracing	Number of cases	Case numbers	Description of ECG changes
A	3	1, 10, 22	Normal ECG
B	6	2, 3, 5, 12, 13, 17	Flat or negative T waves; ST interval frequently depressed. Normal conduction
C	4	6, 7, 15, 20	Slow conduction. Bundle branch block. Prolonged atrioventricular conduction. Frequent broadening of P wave
D	9	4, 8, 9, 11, 14, 16, 18, 19, 21	Tapering high T waves (especially in precordial leads). QRS complex suggestive of hyperpotassaemia

(i) Flat or inverted T waves, and — in many cases — a slight (0.1 to 0.2 mV) depression of the ST interval. Conduction time was normal.

(ii) A conduction disturbance was the characteristic finding. The QRS complex was lengthened (between 0.12 and 0.15 sec.), notched; there was a left bundle-branch block in 3 cases and a right one in one case. The duration of the frequently biphasic P wave was usually prolonged (0.10 to 0.12 sec.), so that a slowing down of atrial conduction had to be assumed. The PQ interval was prolonged in 3 cases (0.20 to 0.22 sec.).

(iii) The most common ECG changes were similar to that seen in hyperpotassaemia. The T wave was pointed, narrow and tall (tent shaped), sometimes the ST interval was also slightly elevated (0.1 mV). It was in the V_1 to V_4 leads that the elevation of the T wave appeared to be most pronounced. The QRS interval, too, was mostly somewhat prolonged (0.10 to 0.11 sec.) without, however, showing signs of bundle-branch block. Atrioventricular conduction time was generally at the uppermost normal limit (0.19 to 0.21 sec.). The P waves were abnormally tall in 50 per cent of the cases, especially in the second and third leads.

We have failed to find a connection between the character and degree of ECG changes on the one side and the results of muscle biopsy on the other.

Figs. 1 to 4 present details of ECG and circulation changes observed in 4 patients.

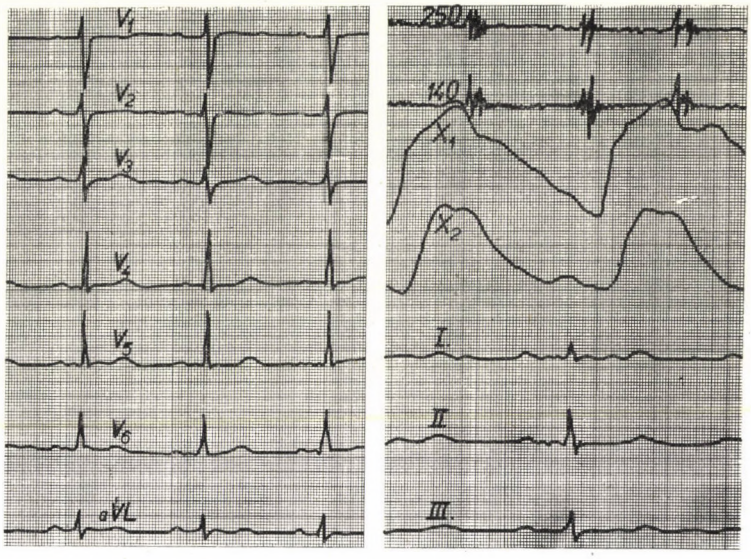

Fig. 1. Gy. K., No. 5: T waves comparatively low in the II and III leads as also in the leads V_2—V_4. Inversion of T wave in lead V_1

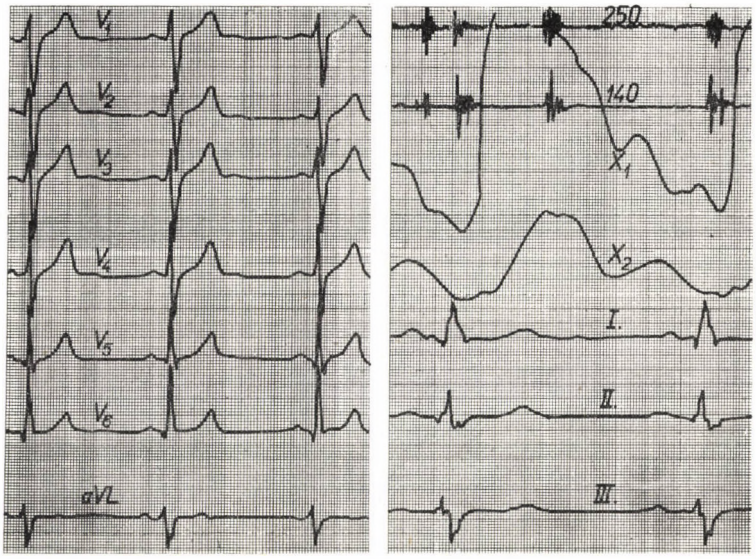

Fig. 2. Á. K., No. 18: T waves tapering and high in the precordial leads V_1—V_6; QRS at the upper limit of normal value (0.10 sec.) ECG of the hyperpotassaemic type

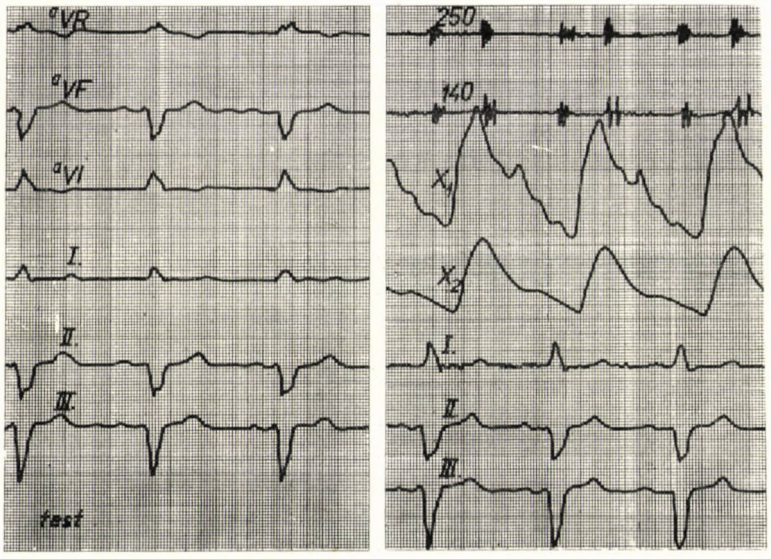

Fig. 3. L. Gy., No. 20: Left bundle-branch block. The second heart sound is split, the first broadened in the phonocardiogram

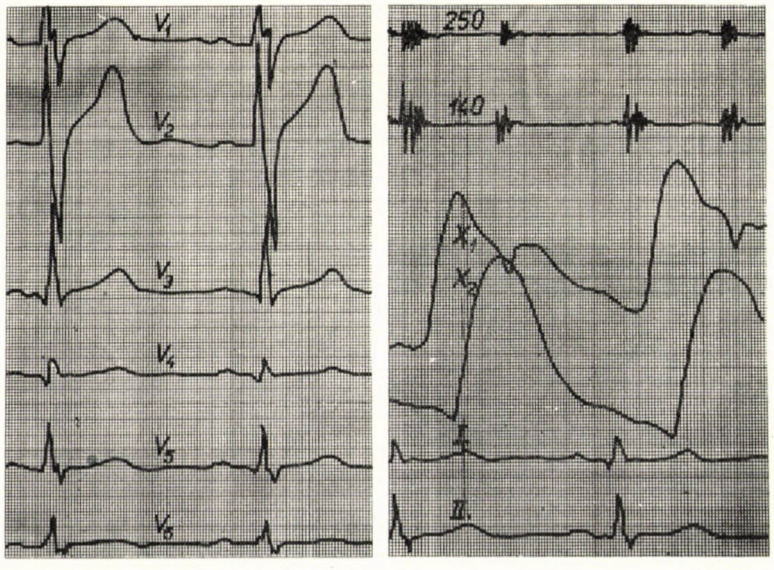

Fig. 4. K. P., No. 14: Left bundle-branch block. Split first and second heart sounds

Discussion

Data regarding circulatory phenomena in connection with progressive muscular dystrophy are fairly scarce. BOAS and LOWENBURG [11] found chronic tachycardia in 7 patients suffering from the disease. GAILANI et al. [12] catheterized the right heart in 12 cases and found an increased right ventricular pressure in 3 cases. KILBURN et al. [13] observed a decreased tidal air volume, diminution of vital capacity, reduced oxygen saturation and elevated pulmonary arterial pressure.

The present findings revealed an increased peripheral resistance and a decrease in stroke volume. The mechanic systole lagged behind the electric systole (Hegglin's energetic dynamic insufficiency). While more numerous, the reports on ECG changes are contradictory. That the ECG of muscular dystrophic patients frequently shows changes is generally recognized. RUBIN and BUCHBERG [4] dissent in this respect; in 17 patients with muscular dystrophy they found slight deviation in the P and Q waves only. PUDDU and MUSSAFIA [14] are also of the opinion that ECG changes are infrequent, especially repolarisation changes.

Yet, most authors observed a high percentage of pathological ECG changes which could not be ascribed to any other cardiac disturbance. The common change was the flattening of the T wave and a depression of the ST interval [6, 9, 15, 16]. Another type of ECG changes, as observed by SCHOTT et al. [17] and MANNING and CROPP [18], consisted in high R and T waves, especially in precordial leads. GAILANI et al. [12] observed right bundle-branch block and delayed right ventricular conduction. Bundle-branch block was observed by TOURNAIRE et al. [15] as well. The findings of SCHOTT et al. [17] and MANNING and CROPP [18] are noteworthy inasmuch as they observed ECG changes only in cases of pseudohypertrophic muscular dystrophy.

The frequent occurrence of pathologic ECG changes in progressive muscular dystrophy has been proved by our findings. Most of our patients were young, and none of them displayed any symptom pointing to congenital malformation or heart disease. The fact that 19 of the 22 patients had pathological ECG changes argues in favour of our statement. The observed changes belonged to 3 main types. (i) Noncharacteristic repolarisation disturbance, a phenomenon observed by most authors [6, 7, 15, 16]. (ii) Disturbance of conduction, a phenomenon observed both by us and other authors in some instances [12, 14]. (iii) A type of changes suggestive of hyperpotassaemia, characterized by pointed high T waves, especially in the precordial leads. The changes observed by SCHOTT et al. [17] as also by MANNING and CROPP [18] presumably belong to this third category. It seems, therefore, that our three types comprise all ECG changes occurring in progressive muscular

dystrophy. According to our observations, there exists no ECG tracing characteristic of pseudohypertrophic muscular dystrophy, nor can we accept the statement that pathologic ECG changes occur only in this form of the disease. Let us add that no author reporting on progressive muscular dystrophy seems to have performed muscle biopsy, although no reliable diagnosis can be made without it. Contradictions in the literature may be due to diagnostic factors of this kind.

Our results have shown that there often occur circulatory disturbances in progressive muscular dystrophy, and that the changes are typical. It should be noted that ECG and circulatory phenomena showed no connection with either the degree of muscle involvement or the result of muscle biopsies. It was remarkable that symptoms pointing to incompensation (dyspnoea, ankle oedema, etc.) were comparatively rare. It is, of course, true that patients suffering from the disease are more or less immobilized so that the circulatory failure may long remain concealed.

As regards the mechanism of the changes, it seems obvious that these are present not only in the skeletal muscles but also in the heart. Yet, cases have been recorded in which post-mortem examination revealed no histological change in the heart, although the ECG had been pathologic, and *vice versa*.

We agree with other authors in that the clinical gravity of the disease is frequently independent of the degree of circulatory changes, so that other factors, too, must be at play. We frequently found energetic dynamic insufficiency with the circulatory changes, a disorder usually associated with disturbances of potassium balance [19]. ECG changes, such as the occurrence of high pointed T waves, a slight elevation of the ST interval and a moderate disturbance of conduction, were frequent in our patients and are likewise frequently associated with disturbances of the potassium balance. There are experiments to show that this balance is often upset in progressive muscular dystrophy, thus it has been demonstrated by means of labelled potassium that both the uptake and release of potassium is reduced in such cases [20, 21]. The potassium content of the skeletal muscles were found to have diminished, while their sodium content augmented [20, 21, 22]. DANOWSKI et al. [23] found a slighter than normal decrease in the potassium level after the administration of insulin and dextrose. Since changes in the K level of the skeletal and cardiac muscles are generally parallel, it seems safe to assume that changes in the potassium content of the heart, that is, changes in the intracellular potassium level, are responsible for the circulatory changes in progressive muscular dystrophy. It is known that both the metabolism and the energy utilization of the muscles are impaired in the disease under discussion. In order to elucidate its mechanism, it is necessary that observations concerning potassium and sodium exchange, circulation and ECG be made in a large number of patients.

Addendum : We studied further 7 cases of progressive muscular dystrophy after this paper had been composed. Cardiac output as determined with the Hamilton Stewart dye dilution method was decreased and the ECG showed pathologic changes in every instance.

REFERENCES

1. Ross, J.: Brit. Med. J. **1,** 200 (1883).
2. Sachs, B., Brooks, H.: Amer. J. Med. Sci. **122,** 54 (1901).
3. Potter, F. C.: N. Y. med. J. **90,** 398 (1909).
4. Rubin, I. L., Buchberg, A. S.: Amer. Heart J. **43,** 161 (1951).
5. Hurwitz, S.: Arch. Neurol. Psychiat. **36,** 1294 (1936).
6. Shank, R. E., Gilder, H., Hoagland, C. L.: Arch. Neurol. Psychiat. **52,** 431 (1952).
7. Berenbaum, A. A., Horowitz, W.: Amer. Heart J. **51,** 622 (1956).
8. Frank, K.: Orvosképzés 67—72 (1939).
9. Kiloh, L. G., Nevin, S.: Proc. roy. Soc. Med. **44,** 694 (1951).
10. Wezler, K., Böger, A.: Ergebn. Physiol. **41,** 297 (1939).
11. Boas, E. P., Lowenburg, H.: Arch. intern. Med. **45,** 376 (1952).
12. Gailani, S., Danowski, T. S., Fischer, D. S.: Circulation **17,** 583 (1958).
13. Kilburn, K. H., Sieker, H. O., Heyman, A., Eagan, J.: 31st Scientific Session of the American Heart Association, San Francisco, 1958.
14. Puddu, V., Mussafia, A.: Arch. mal. Coeur **32,** 958 (1939).
15. Tournaire, A., Fournie, G., Blum, J., Semet, J.: Arch. mal. Coeur **48,** 738 (1955).
16. Weisenfeld, S., Messinger, W. J.: Amer. Heart J. **43,** 170 (1952).
17. Schott, J., Jacobi, M., Wald, M. A.: Amer. J. med. Sci. **229,** 517 (1955).
18. Manning, G. W., Cropp, G. J.: Brit. Heart J. **20,** 416 (1958).
19. Elster, K., Wallner, A.: KreislaufForschg. **45,** 586 (1956).
20. Baker, N., Bladh, W. H., Hart, P.: Amer. J. Physiol. **193,** 530 (1958).
21. Shy, G. M., Cummings, D. J., Berg, L., Horvath, B.: J. appl. Physiol. **8,** 22 (1955)
22. Young, H. L., Young, W., Edelman, I. S.: Amer. J. Physiol. **197/2,** 487 (1959).
23. Danowski, T. S., Gillespie, H. K., Egan, T. J.: J. Dis. Child. **91,** 429 (1956).

Dr. Ferenc Solti, Dr. Ernő Zádory } Budapest, VIII. Korányi S. u. 2/a I. sz. Belklinika, Hungary

Dr. György Bekény, Budapest, VIII. Balassa u. 8. Idegkórtani Klinika, Hungary.

RADIATION EFFECT ON ELECTROLYTE AND WATER BALANCE, PARTICULARY ON EXTRACELLULAR ION SHIFTS

By

Z. ZSEBŐK and GY. PETRÁNYI jr.

RADIOBIOLOGICAL RESEARCH LABORATORY, FIRST DEPARTMENT OF SURGERY, UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received October 4, 1961)

After a brief survey of the literature on radiation-induced electrolyte and water disturbances in living organisms, primarily mammals, investigations into the changes in the total sodium and potassium contents of the extracellular fluid and into urinary salt excretion during the 3-to-5-day effect have been reported. The results have revealed the occurrence of marked ionic shifts. The possible explanation and significance of the changes have been discussed.

A number of metabolic processes are known to be interrelated with certain definite electrolyte concentrations. For example, in the cellular depolarisation phase when energy is released by processes such as glycogen or protein decomposition or adenosinetriphosphate (ATP) synthesis, the relative intracellular potassium concentration is low and the sodium concentration high. Conversely, in the repolarisation phase the anabolic processes and the decomposition of ATP require a higher potassium and a relatively low sodium concentration. An alteration in the molecular concentration of the potassium ions suffices to inhibit or activate the afore-mentioned metabolic processes. A similarly close interaction has been found to exist between enzymatic functions and certain anions and cations, respectively [4, 9, 26, 29, 30, 43, 44, 46].

The electrolyte concentrations required for the intracellular metabolic processes are ensured by the active and passive functions of the cell membrane. The active function, so-called sodium pump, is an energy consuming process; the passive function rests upon changes in the cellular membrane potential. Under physiological conditions the two processes together keep up the difference between the intracellular and the extracellular ion concentrations and maintain the constancy of a balanced ion exchange [4, 7, 8, 26, 28, 29, 58].

Equally great is the biological significance attaching to the extracellular ion content of the organism. The intracellular and extracellular fluids differ in electrolyte concentration but the cell membrane separating the two systems does not constitute an impassable barrier between them. Should some pathological process bring about a change in the extracellular ion content, the intracellular content will not fail to show after some time the effect of that change. The organism persistently endeavours to keep the

extracellular electrolyte concentrations at constant levels, and in maintaining them, a role of paramount importance is played by the ion turnover in the kidneys, bones, gastrointestinal tract, the skin and the lung. While not wishing to discuss the integration of the ion turnover in detail, we should nevertheless like to point out the considerable extent to which the ion balance of the organism is governed by the neuroendocrine system. Impaired functioning of the above organs, by interfering with the composition of the extracellular space, may upset the electrolyte balance in the organism [4, 9, 14, 23, 24, 25, 28, 29, 32, 45, 47, 49, 58].

Even this brief survey will suffice to show how wide the area is over which the elctrolytes via the various regulating organ systems render the organism accessible to the complex effects of ionizing radiation. The syndromes produced by ionizing radiation lend themselves to discussion in two groups, viz.:

1. Disturbances of the active and passive ion transport through the cell membrane resulting primarily in changes of the intracellular electrolyte concentration.

2. Functional disturbances of the individual regulatory organs due to radiation sickness which, generally by increasing the rate of ion loss, produce secondary shifts in the ion content of the organism.

As regards the first group, HARRISON, BRUCE and STAPLETON [27] have recently observed an increased loss of potassium from E. coli as a result of irradiation with high doses. KWAN-HSU and DOMNER [37], and SWIFT and TAKETA [54] reported similar results for yeast. ROTHENBERG [51] noticed increased Na isotope uptake by axons in the fish. In frozen erythrocytes, LESSLER [cit. 43] observed simultaneously with an increase of the potential a transient increase in K^{42} uptake followed by marked K outflow. In careful studies MORCZEK [42, 43] showed that irradiation of human red blood cells in vitro promoted the loss of potassium from them. Disturbing the permeability of the cell membrane in this manner eliminates the difference between the intracellular and extracellular concentration of ions.

The results of the experiments of CASTER and ARMSTRONG [12] with different types of tissue, of ELLINWOOD, WILSON and COON [18] with mammalian hearts, and of BRESCIANI, DOSE and RAJEWSKY [11] with surviving organs from rats, leave no reasonable doubt that after intensive irradiation there will be loss of intracellular potassium and that the cell membrane will lose its capacity to act as a sodium pump. The metabolic changes associated with the alterations in the ion content correspond to those observed in the depolarization stage of the cell [10, 21, 31, 38]. BERGEDER [6, 7, 8] considers the change of the membrane potential and the energy-consuming metabolic processes instrumental in the transport of ions, to be responsible for the ionic shifts.

Concerning the second group of syndromes, it is difficult to classify the results according to the organs involved, the pathologic phenomena being closely interconnected. It therefore seems expedient to discuss the secondary shifts of ions as syndromes arising in response to different dose levels. The present work is only concerned with the shifts of sodium and potassium ions in the gastrointestinal syndrome.

Sodium. In a series of experiments JACKSON, RHODES and ENTENMAN [33] showed that the loss of sodium from the intestinal tract was the highest on the third postirradiative day, and that the loss reduced the organism's total sodium by 1.50 mEq. At the same time, they presented evidence by intraperitoneal dialysis that a loss of 1.45 mEq of sodium was bound to be fatal. They found the cause of the loss, in the inhibition of intestinal sodium reabsorption excreted with the bile. QUASTLER [47] and CONARD et al. [13] likewise observed that X-irradiation deprived the intestinal wall of its capacity to act as a barrier and thereby caused the organism to lose electrolytes and water. CURRAN et al. [15] observed in the isolated ileum a loss of sodium on the third postirradiative day; they explained this for the greater part by the cessation of the barrier action of the desquamated epithelial layer, and partly by impaired absorption. At any rate, there appears to be little doubt that the "gastrointestinal death" is primarily due to a loss of sodium and water.

Potassium. CASTER and ARMSTRONG [12] and JACKSON and ENFTENMAN [32] have established fact that the total loss of potassium during the first four days after irradiation is considerable, yet not as disastrous as to be the sole cause of death. It is the heaviest in the muscle and connective tissues. The amount of potassium excreted with urine on the first and third days is, on an average, 27 per cent higher in irradiated than in control animals [12, 32, 33, 40]. The early postirradiation disturbances in the ion-secreting activity of the kidneys still await elucidation [35, 41].

Chlorine. The above quoted authors observed chlorine to start migrating to the stomach three hours after irradiation, with alkalosis and a decreasing chlorine level showing twelve hours later in the blood plasma. The Na/Cl-ratio changed in muscle and connective tissue, and there was a negative chlorine balance within the body. According to BAKER and HUNTER [1, 2], enhanced gastric secretory activity is one of the causes of intensified chlorine migration.

Since the electrolyte and water balances cannot be discussed except in close correlation with each other, we must briefly dwell upon the atter subject. ENTENMAN, EVERSOLE and JACKSON observed polydipsia and polyuria in mammals one hour after exposure to 1500 r whole-body irradiation, the cause of which has still to be explained [19, 33]. SOBERMANN et al. [53], VÁRTERÉSZ et al. [57], SWIFT and TAKETA [54], and a number

of other authors observed a decrease in plasma volume in several species of irradiated animals. Changes in the extracellular space, too, are possible, but of these we have as yet no clear conception. Following local X-irradiation in large doses, WILDE and SHEPPARD [52, 59], and DARDEN [52], noticed in muscle an increase of the extracellular water space which probably resulted from an increased permeability of the blood capillaries.

As regards the neuroendocrine system, one point of considerable interest has to be mentioned. In mammals given a lethal dose of X-irradiation, a biphasic reaction passing through the adrenals is observable, from which several authors have concluded to an enhanced adrenal cortical activity in both the early and the late phase of the reaction [4, 5, 20, 24, 25, 48, 50, 55]. Disturbed salt and water balances, too, may be related to disturbed mineralocorticoid secretion by the adrenal cortex [24, 25, 45]. Yet, the problem still is an intricate one. There is, for instance, the frequent discrepancy between synthesis and secretion of adrenocortical hormones in the observed aspecific neuroendocrine reaction (DÁVID et al., 16). This point will be discussed in detail in a subsequent paper.

Even this brief and incomplete survey of the pertaining literature clearly points to certain shifts and considerable loss of electrolytes from the organism exposed to irradiation. The question presents itself what changes in the total ion content of the extracellular space are produced by the radiation-induced shifts of intracellular ions and the increased loss of ions from the kidneys and the gastrointestinal tract. Many authors agree that radiation sickness does not give rise to significant changes in the concentration of ions in the plasma [4, 12, 22, 33, 43, 47, 53]. The organism, by bringing its regulatory mechanisms into play, obviously tries to maintain at any cost the constancy of the electrolyte concentrations which it needs for its biological functioning.

For this reason it was thought necessary to study the alterations taking place not only in the serum ion concentrations but also in the total extracellular ion content. In addition, the changes in the amount of ions lost through the kidneys were registered. Sodium and potassium being the electrolytes of commanding importance, the present paper reports only results concerning these two.

Materials and methods

Including all preparatory examinations, 420 rats of the Wistarstrain, of either sex, 4 to 6 months old, and weighing 150 to 300 g, were used. Throughout the experiments the animals were offered the same amount of food of the same composition. Water was not restricted. Feeding occurred once a day so that examinations and tests could be made with the animals near the fasting state. Whole-body irradiation was made with a Stabilipan (Siemens) X-ray apparatus at 200 kV, 20 mA, 0.5 mm Cu filter, 35.0 r/min. The dose given was 1500 r. The HVL was 1.0 mm Cu. Doses were controlled on each occasion using a Siemens Universal

dosimeter. For irradiation the animals were placed into plexiglass cages divided into compartments.

The following method was employed to eliminate the influence on the serum sodium and potassium values of metabolic changes in red blood cells, platelet decomposition accompanying clotting, and contamination by interstitial fluid. The rats were bled to death by heart puncture under ether anaesthesia. Instead of a syringe a closed suction system conducted the blood straight into a centrifugal tube prewarmed to the animal's body temperature. Sodiumfree heparin was used as anticoagulant and to enhance the resistance of damaged erythrocytes [9]. The blood was centrifuged immediately on one occasion for 10 minutes at 900 to 1000 r. p. m. Only one or two of the sera obtained in this way showed haemolysis; these were discarded. In this manner it was also possible to reduce to a negligible level the potassium contamination released by damaged erythrocytes. In our view the haemolysis observed frequently after high dose irradiation is a secondary phenomenon probably due to technical error; red blood cells damaged by radiation are far more sensitive than intact ones to interference by mechanical force, temperature differences, standing for longer periods of time, etc.

Sodium and potassium were estimated by flame photometry. Urine was collected individually in 24-hour portions.

The extracellular space was determined with inulin administered on a single occasion to animals with their renal hili ligated. (A detailed description of the method will follow in a subsequent paper.) It showed the extracellular space of intact rats to be on the average 17.1 per cent, with a maximum scatter of ± 1.1 per cent.

The total sodium and potassium content of the extracellular space was calculated from the serum levels and water space determined at the same point of time.

Results

The experiments were carried out in 5 parallel series, with 20 experimental and 20 control animals in each one. Interventions and bleedings were performed at 1, 6, 12, 24, 48, and 72 hours after irradiation. In the following the respective mean values of the five series will be given.

Serum sodium and potassium concentrations. The values obtained for the concentrations of sodium and potassium at 1, 6, 12, 24, and 48 hours following irradiation, varied between limits within the scatter. The deviations noted were too slight to permit conclusions. Paradoxically, the level of both sodium and potassium was decreased at 72 hours, by which time gastrointestinal signs and symptoms, and dehydration with haemoconcentration were predominating. It will be outlined later why in that case the changes did not truly reflect the actual ionic shifts. The relative data are listed in Table 2.

Extracellular fluid. In the first 12 hours after irradiation no characteristic changes were noted in the extracellular fluid, but its tendency to instability was unmistakable. This was particularly remarkable when considered in relation to the limited scatter in the controls. The changes taking place in the extracellular fluid in this first 12-hour period will be more fully studied in future experiments to be carried out under rigorous conditions and employing selective methods. At 24 hours after irradiation the extracellular fluid was found to have reached the 20-per cent level whence it gradually sank to 10 per cent at 72 hours, with the gastrointestinal syndrome developing at about the same rate. In a rat weighing 200 g the 10-per

Table 1

Extracellular space in rats after 1500r whole-body irradiation

	Per cent	Percentage scatter
Prior to irradiation and controls	17,1	±1,1
After irradiation		
1^h	18,9	±1,8
6^h	16,8	±2,2
12^h	14,5	±0,9
24^h	19,9	±1,3
48^h	15,6	±1,2
72^h	9,8	±0,8

cent level represents a 10 to 14 ml loss of fluid. Clinical observations show this to be an acceptable value, afforded support by dehydration, decrease in blood volume, and increase in the exudate accumulating in the gastro-intestinal tract.

Total sodium and total potassium contents of the extracellular fluid. Unlike that of the concentrations, the study of the amounts of extracellular sodium and potassium brought a number of disturbances to light. The values listed in Table 3 are of course not identical with the absolute values, but as they approach them rather closely, it is safe to regard them as true reflections

Table 2

Serum sodium and potassium levels in rats after 1500r whole-body irradiation

	Sodium	Potassium
	mEq/l	
Prior to irradiation and controls	150 ± 4,12	4,51 ± 0,34
After irradiation		
1^h	136 ± 2,31	4,23 ± 0,25
6^h	146 ± 3,82	4,36 ± 0,41
12^h	148 ± 1,12	4,56 ± 0,31
24^h	143 ± 4,00	4,51 ± 0,23
48^h	142 ± 2,11	3,82 ± 0,31
72^h	138 ± 0,91	3,13 ± 0,10

Table 3

Changes in total extracellular sodium and potassium in rats after 1500r whole-body irradiation

	Sodium			Potassium		
	mg/100 g	% deviation	P value	mg/100 g	% deviation	P value
Prior to irradiation and controls	58,6 ± 4,00			2,98 ± 0,20		
After irradiation						
1^h	59,6 ± 4,56	+ 1	$P > 0,5$	3,15 ± 0,24	+ 6	$0,2 < P < 0,5$
6^h	57,5 ± 8,59	— 2	$P > 0,5$	2,86 ± 0,13	— 4	$P < 0,5$
12^h	49,3 ± 2,59	—16	$P < 0,01$	2,58 ± 0,27	—13	$0,01 < P < 0,02$
24^h	63,6 ± 5,05	+ 8	$0,1 < P < 0,2$	3,52 ± 0,30	+18	$P \ll 0,01$
48^h	51,5 ± 4,97	—12	$0,02 < P < 0,05$	2,34 ± 0,17	—21	$P \ll 0,01$
72^h	34,6 ± 2,40	—41	$P \ll 0,01$	1,19 ± 0,08	—60	$P \ll 0,01$

of the extracellular ionic changes. Experiments carried out under specific conditions in normal and control animals showed that the extracellular fluid contained 58.6 (± 4.0) mg per 100 g of total sodium and 2.98 (± 0.2) mg per 100 g of total potassium. The postirradiation changes are presented in Table 3. The changes in total sodium practically paralleled those in total potassium. In neither were the changes substantial until the 12th hour after irradiation when a significant drop in the total ion content took place ($P < 0.01$). At 24 hours, a slight rise in the total sodium could be observed ($0.1 < P < 0.2$), but in view of its not having been significant statistically, it seems correct to state that a gradual decline in the first 48 hours developed into an abrupt fall at 72 hours ($P \ll 0.01$). The changes in total potassium presented a more characteristic picture: a rise observed at 24 hours ($P \ll 0.01$) was followed by a steep decline lasting until the 72nd hour ($P \ll 0.01$). Immediately before death, the total sodium content of the extracellular fluid was found to be 41 per cent less, and the total potassium content 60 per cent less, in the experimental than in the control animals (Fig. 1).

Amount of urinary sodium and potassium. The postirradiation changes in urinary sodium and potassium contents are expressed numerically in Fig. 2. Numerical values have been given as the figures for concentration are of informative value only when equal amounts of urine are excreted or after a water load. Spontaneous postirradiation urine excretions, on the other hand, vary widely in amount. In our present experiments the average extremes were polyuria with 22.1 ml/100 g on the first day, and oliguria with 0.4 ml/100 g on the third day. The changes observed in total urinary sodium and potassium

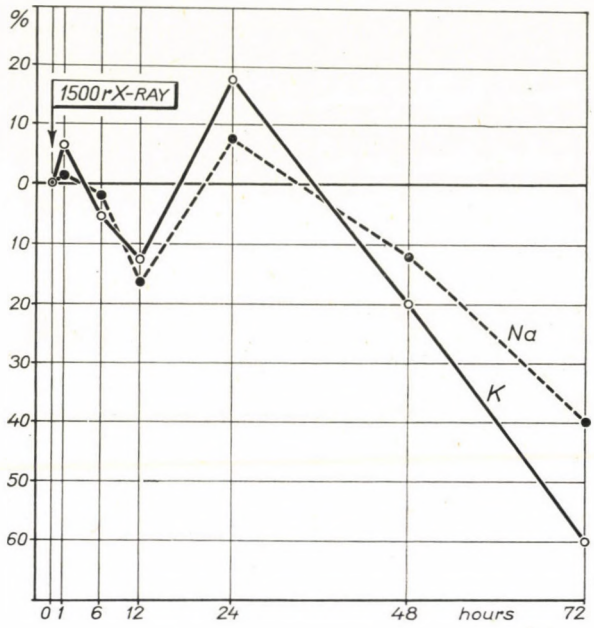

Fig. 1. Changes in total extracellular sodium and potassium in rats after 1500 r whole-body irradiation

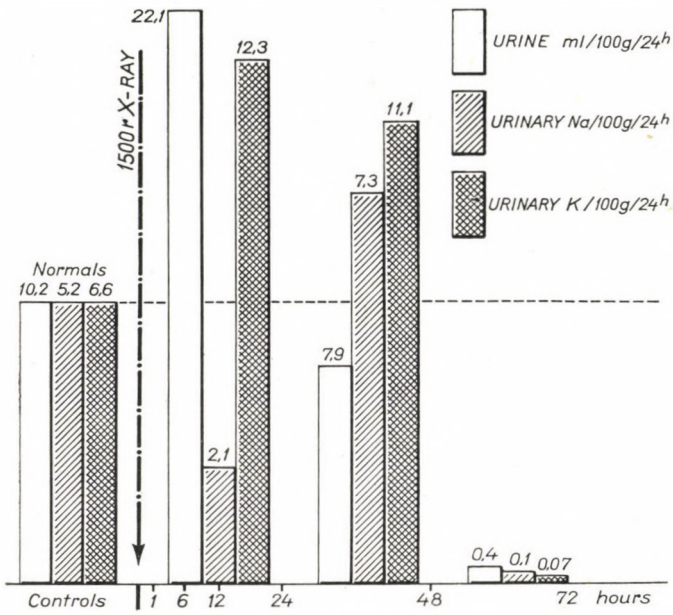

Fig. 2. Total urinary sodium and potassium excretion in rats after 1500 r whole-body irradation

excretion in 24 hours are represented in Fig. 2, which shows that in the first 24 hours after irradiation, potassium excretion increased to almost the double, and sodium excretion decreased to less than half, the normal level. On the third day, the excretion of both sodium and potassium dropped to a minimum. This may have been due to the simultaneous dehydration. Contrary to our findings, JACKSON and ENTENMAN [32] reported a further increase in potassium excretion occurring on the third day. A possible explanation for the conflicting results seems to be that they have based their opinion upon concentration values which were of course deceivingly high when the 24-hour amount of urine was about one hundredth of the amount before irradiation.

Discussion

The values obtained for extracellular sodium and potassium and their amounts in urine, are not sufficient for drawing conclusions, but if considered in their relations to the relevant data in the literature, they may still give some information concerning the electrolyte disturbances induced by radiation injury.

During the first 24 postirradiation hours the electrolyte and water balances are unstable and the following processes develop. Polyuria, polydipsia [4, 24, 25, 33, 45], enhanced gastric secretion [1], and instability of the extracellular space with a tendency to expand are noticeable. Instability of the extracellular space and its tendency to expand may be phenomena concomitant to polydipsia and polyuria, which are still imperfectly known conditions or they may result from increased permeability of capillary walls [4, 52, 57]. As to the electrolyte balance, in the first 24-hour period there is a rise in the total sodium and potassium contents of the extracellular fluid concurrent with decreased sodium and increased potassium excretion. The rise in extracellular potassium probably derives in part from the release of cellular potassium due to changed permeability conditions and partly from disintegrated radiation-susceptible tissue elements. The kidneys excrete the extracellular potassium at a higher than the usual rate. Certain signs — such as a rise in extracellular sodium, and increased urinary potassium with decreased urinary sodium — indicate enhanced adrenocortical activity or the entrance of excess mineralocorticoids into the blood. In our view these secondary signs must be more apparent than real and do not permit conclusions as to adrenocortical activity; such activity cannot be expected to follow whole-body irradiation of more than 1000 r.

On the second day after irradiation both, the water and the electrolyte balance began shifting in the negative direction: the extracellular space contracted to 15.5 per cent, the total content of sodium dropped by 12 per

cent, and that of potassium by 21 per cent. This shift was the consequence of a deficient uptake due to anorexia and of the organism's losing its capacity for retaining electrolytes and water, the latter manifesting itself with an increased excretion of ions.

It has already been mentioned that according to JACKSON and ENTENMAN [32] disturbances in enteral ion turnover are caused by the failure of the sodium excreted with bile to reabsorb in the small intestine. Since then GOODNER [cit. 15] has shown that at the stage in question sodium reabsorption is not yet inhibited. The conclusion which seems to follow from the low ionic content of the extracellular fluid, is one of an imperfect ion turnover which finds its explanation in that, retarded by slow and reversed peristalsis, the intestinal juice is unable to reach the large intestine where at this time the sodium could still be reabsorbed.

At around 72 hours, the electrolyte and water balance is suddenly upset; the extracellular space is found to have contracted to 10 per cent, the total sodium to have decreased by 41 per cent, and the total potassium by 60 per cent. Were one guided by concentration figures alone, one would be mislead by these values barely showing any differences from normal. After the 67th postirradiation hour, the epithelial lining of the gut starts necrotising, the intestinal wall becomes gradually denuded [15, 47], and completely loses both its active and passive capacity for transporting ions [15, 33, 47]. The result is unimpeded inpour of water and ions from the extracellular space into the intestinal tract. This observation of CURRAN et al. [15] is clearly borne out by the abrupt drop in the total ion content of the extracellular fluid observed by us. The marked salt deficiency and dehydration results in decreased diuresis and urinary ion excretion.

The salt and water loss outlined in the foregoing appears to come into play as one of the direct aetiologic agents in gastrointestinal death. Of course, the part of the electrolyte and water disturbances in the wide-ranged damaging action of ionizing radiation is neither primary nor exclusive. Nevertheless, among the syndromes elicited by irradiation at different dose levels there are some in which the role played by these disturbances cannot be neglected.

REFERENCES

1. BAKER, D. G., HUNTER, C. G.: Radiat. Res. **6,** 660 (1958).
2. BAKER, D. G., HUNTER, C. G., SCHÖNBAUM, E.: Radiat. Res. **12,** 409 (1960).
3. BÁLINT, P.: Klinikai Laboratóriumi Diagnosztika, Művelt Nép, Budapest, 1955.
4. BACQ, Z. M., ALEXANDER, P.: Fundamentals of Radiobiology. Pergamon Press, London, 1961.
5. BACQ, Z. M., SMELIK, D. S., GOUTIER-PIROTTE, M., RENSON, J.: Brit. J. Radiol. **394,** 618 (1960).
6. BERGEDER, H. D., HOCKWIN O.: Strahlentherapie **113,** 207 (1960).
7. BERGEDER, H. D.: Strahlentherapie **114,** 406 (1961).
8. BERGEDER, H. D.: Strahlentherapie **115,** 91 (1961).

9. Boda, D.: Folyadék és elektrolit therápia. Medicina, Budapest, 1960.
10. Bowers, J., Scott, K. G.: Proc. Soc. Exp. Biol. (N. Y.) **78,** 645 (1951).
11. Bresciani, F., Dose, K., Rajewsky, B.: Naturforsch. **14,** 158 (1959).
12. Caster, W. O., Armstrong, W. D.: Radiat. Res. **5,** 189 (1956).
13. Conard, R. A., Cronkite, R. P., Brecher, G., Strume, J.: J. appl. Physiol. **9,** 227 (1956).
14. Curran, P. F., Solomon, A. K.: J. gen. Physiol. **41,** 143 (1957).
15. Curran, P. F., Webster, E. W., Hovsepian, J. A.: Radiat. Res. **13,** 369 (1960).
16. Dávid, G., Farádi, L., Tanka, D.: Honvédorvos, **2,** 154 (1961).
17. Dunjic, A., Maisin, J., Maldague, P., Maisin, H.: Radiat. Res. **12,** 2 (1960).
18. Ellinwood, L. E., Wilson, J. E., Coon, J. M.: Proc. Soc. exp. Biol. (N. Y.) **98,** 740 (1958).
19. Edelman, A., Eversole, W. J.: Am. J. Physiol. **163,** 709 (1950).
20. Gerber, G.: Strahlentherapie **110,** 510 (1959).
21. Ginsburg, J. M., Ulmer, D. P.: U. S. A. Marine Res. Lab. Rep. No. 339 (1958).
22. Goodmann, R. D., Vogel, M.: Amer. J. Physiol. **175,** 29 (1953).
23. Glynn, I. M.: Progr. Biophys. **8,** 241 (1957).
24. Gorizontov, P. D., Protasova, T. N.: Arkh. Path. **4,** 3 (1960).
25. Grodzenskij, A. E.: Med. Radiologija 4/5, 77 (1959).
26. Hadorn, W.: Regensburg. Jb. ärztl. Fortbild. **6,** 424 (1961).
27. Harrison, A. P. jr., Bruce, A. K., Stapleton, G. E.: Proc. Soc. exp. Biol. (N. Y.) **98,** 740 (1958).
28. Heftner, E.: Münch. med. Wschr. **103,** 13 (1961).
29. Heidenreich, O.: Dtsch. med. Wschr. **12,** 10 (1961).
30. Hertel, H.: Materia Medica Nordmark. **6,** 4 (1954).
31. Hick, S. P., Wright, K. A.: Arch. Path. **61,** 226 (1956).
32. Jackson, K. C., Entenmann, C.: Radiat. Res. **10,** 67 (1959).
33. Jackson, K. C., Rhodes, R. L., Entenmann, C.: Radiat. Res. **8,** 361 (1958).
34. Kahn, J. B., Furth, J.: Blood **7,** 404 (1952).
35. Kisvárdai, Gy.: Orv. Hetil. **20,** 913 (1961).
36. Kovách, A.: Kísérl. Orvostud. Vizsgáló Módszerei. Akadémiai Kiadó, Budapest, 1954.
37. Kwan-Hsu, Domner, J.: Annual Meeting of the Radiation Res. Society, San Francisco, 1960.
38. Lautit, J. F.: Biological action of radiation. Lect. sci. Basis Med. **1,** 379 (1952).
39. Mason, W. B., Coulter, M. P., Furth, F. W.: Univ. Rochester Atomic Energy Project UR-189, 16 (1951).
40. Mey, U.: Z. inn. Med. **15,** 255 (1961).
41. Michailov, V., Karpatov, R.: Radiat. Biol. Radiat. Ther. **3,** 345 (1960).
42. Morczek, A.: Fortschr. Röntgenstr. **88,** 584 (1958).
43. Morczek, A.: Die ionisierenden Strahlen und der Haushalt anorganischer Kationen. VEB. G. Thieme, Leipzig, Stuttgart 1957.
44. Naegeli, H.: Helv. med. Acta Suppl. **20,** 2 (1951).
45. Pentz, E. I.: Proc. Soc. exp. Biol. (N. Y.) **96,** 3 (1957).
46. Peters, G.: Nebennierenrinden-Incretion und Wasser-Elektrolithaushalt. G. Thieme Stuttgart 1960.
47. Quastler, H.: Radiat. Res. **4,** 303 (1956).
48. Rogyionov, V. M., Orlova, L. V., Tuul, B.: Problemü Endocrinologii: Gormonotijer **8,** 33 (1960).
49. Rajewsky, B.: Strahlendosis und Strahlenwirkung. Thieme, Stuttgart, 1956.
50. Rajewsky, B.: Strahlentherapie **100,** 5 (1956).
51. Rothenberg, M. A.: Biochem. biophys. Acta **4,** 96 (1950).
52. Sheppard, C. W., Darden, E. B.: Fed. Proc. **17,** 148 (1958).
53. Sobermann, R. J., Keating, R. Pl., Maxwell, R. D.: Amer. J. Physiol. **164,** 450 (1951).
54. Swift, M. N., Taketa, T.: Radiat, Res. **8,** 516 (1958).
55. Teller, W., Starb, W.: Strahlentherapie **111,** 107 (1960).
56. Várteresz, V., Fráter, K., Kálmán, E., Wald, B.: Strahlentherapie **105,** 467 (1958).
57. Várteresz, V., Fráter, I., Wald, B.: Kísérl. Orvostud. **8,** 34 (1956).
58. Wilbrandt, W.: Regensburg. Jb. ärztl. Fortb. **8,** 413 (1961).
59. Wilde, W. S., Sheppard, C. W.: Proc. Soc. exp. Biol. (N. Y.) **88,** 249 (1955).

Zoltán Zsebők
Győző Petrányi } Budapest VIII., Üllői ut 78, Hungary

ELECTROCARDIOGRAPHIC CHANGES ASSOCIATED WITH HERPES ZOSTER

By

I. PASTINSZKY and I. KENEDI

HUNGARIAN ARMY MEDICAL CORPS

(Received March 20, 1962)

In 26 patients, mostly young males, suffering from herpes zoster, in most cases in the thoracic segments, electrocardiographic changes have been observed. The changes deteriorated and improved parallel to the course of the disease. The incidence of the changes was significantly higher in grave cases, i.e. where several segments were affected, than in those of simple herpes zoster. The disease may give rise to a pathologically deep Q-wave which sometimes becomes chronic without concomitant cardiac symptoms. The localisation of herpes most frequently associated with an abnormal ECG is at the segments Th_2 through Th_7.

Zosteric cardiopathy may be (*i*) of neural origin when it is caused by a disturbance of sympathetic innervation due to ganglionitis; (*ii*) of vascular origin when it is due to a hyperergic vascular disorder of the affected segment, similar to polyarteriitis nodosa (FEYRTER). Histological proofs of the latter theory are still lacking.

Introduction

Herpes zoster is an acute infectious disease characterized by the inflammation of one or more posterior spinal or cerebral ganglia (simple or hemorrhagic or necrotic ganglionitis), and the painful inflammatory lesions of grouped vesicles on the skin or mucous membranes along the sensory nerves originating from the affected ganglion. In addition to the peripheral, the sympathetic nervous system seems also to be involved in the cutaneous changes.

While there exist a few reports on the cardiovascular manifestations of herpes zoster, in the pertaining literature we have found no data concerning electrocardiographic changes in connection with the disease.

Material and method

Electrocardiograms were made mostly in cases of thoracic herpes at admission, at the regression of the condition between 7 and 14 days, and — provided the ECG showed anomalies — one year after healing of the process. Standard limb leads, unipolar limb leads and six precordial leads were used. Of the 26 observed patients (24 males and 2 females) most were 20 to 22 years old, one was 13 and two were above 40 years of age. Organic heart disease was excluded by physical examination, chest X-rays and laboratory tests. Before being examined, the patients received no drug. They were divided in two groups according to the gravity of the process. The first group consisted of 17 patients who had a mild form of the disease extending only to one or two segments; the second group included 9 subjects whose herpes extended to more than two segments or the contents of the vesicles were hemorrhagic. The gravest form of the disease was observed in a female patient of 66 years; it extended over four segments and gave rise to necrosis healing with scars (Fig. 1).

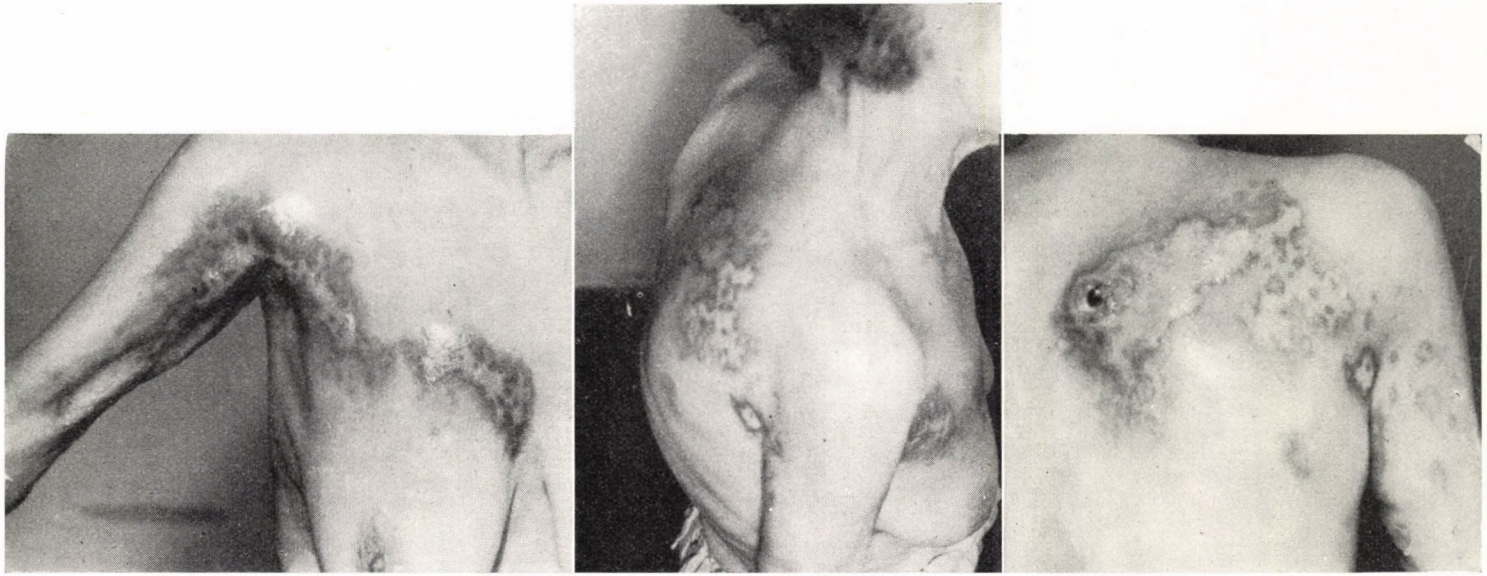

Fig. 1. Herpes zoster extending to four segments and healing with scar in 66-year-old female patient

Results

In 14 of the 26 patients, the ECG showed pathologic changes. These consisted in depressed ST interval or considerably flattened T-wave in 12 cases, and in the presence of a deep Q-wave in 2 instances. Altogether, 60 recordings were made. In four cases, the changes manifested themselves in the second week; the cardiac disorder would have remained unrecognized had we contended ourselves with a single electrocardiogram at admission. The precordial leads showed deviation in four cases, and the aV_L lead in two instances; these changes disappeared after the disease was over, a proof of the ECG changes and the herpes having been interconnected.

Development of ECG changes, their aggravation, and normalisation showed a course parallel to that of the disease. Case No. 5 is a good example in this respect. This patient, a man of 22 years, developed groups of vesicles with seropurulent contents over three segments on the left side (Th_2—Th_4) and the inner side of the left forearm (segment Th_1). The T_2 wave was flat in the first ECG, taken after 2 days; it was negative and the T-wave in the leads $V_{5—6}$ was depressed in the ECG taken 6 days later. Depression of the T-wave was no longer perceptible in the precordial leads when a new ECG was made 9 days later. Wave T_2 was isoelectric after another 5 days. The ECG was normal at the follow-up examination performed after 14 months (Fig. 2).

It was in two cases that an observation of the development of ECG-changes made it evident that the depression of the Q-wave had been induced by the herpes. Case No. 17 is an example in this respect. S. T., a 30-year-old male patient, was admitted December 29, 1960, on account of a belt-like, largely hemorrhagic herpes, which extended to segments Th_5, Th_6 and Th_7 on the left side. A small Q-wave was observed in the lead aV_L and the bithoracal lead D on December 30. A Q-wave became perceptible in the first limb lead and the lead V_5 four days later. After further 3 days there was no essential change. At the follow-up examination 10 months later, there were pathologic Q-waves in the leads I, aV_L and D (Fig. 3).

The last case is worthy of especial note because it shows how easily it may happen that a pathologic Q-wave, observed accidentally, is regarded as a sign of an earlier more or less symptom-free cardiac infarct. The two cases make it clear that herpes zoster, while not causing serious heart trouble, may give rise to pathological Q-waves. If there is such a wave in the ECG, the patient should be questioned about a herpes zoster in the history.

It was likewise by way of a control ECG, taken after the full recovery of a female patient 66 years of age, that the anomalous ECG (depression of the ST segment in the first lead and leads $V_{4—5}$; elevation of the same in lead aV_R) was found to be not of coronary-sclerotic origin but due to a grave herpes which had extended over three thoracic segments.

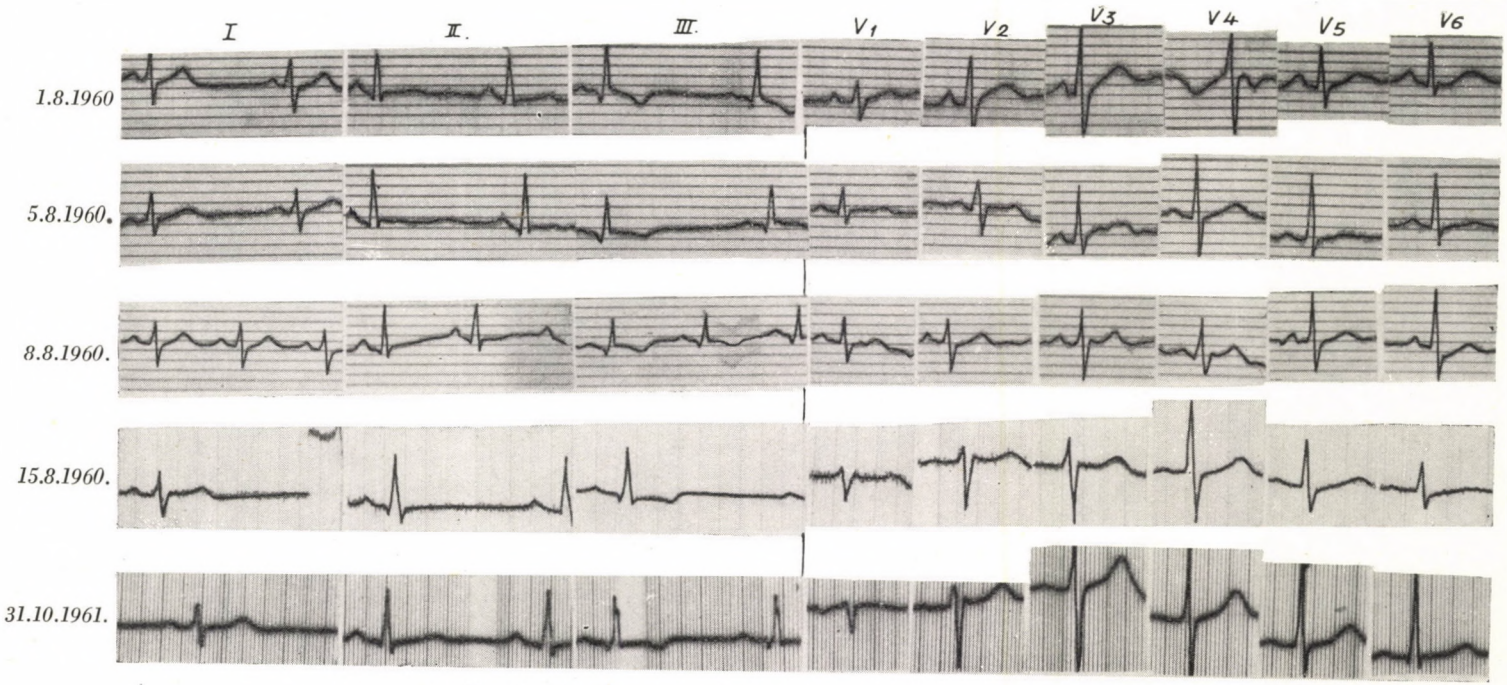

Fig. 2. The dynamics of ECG changes in herpes zoster

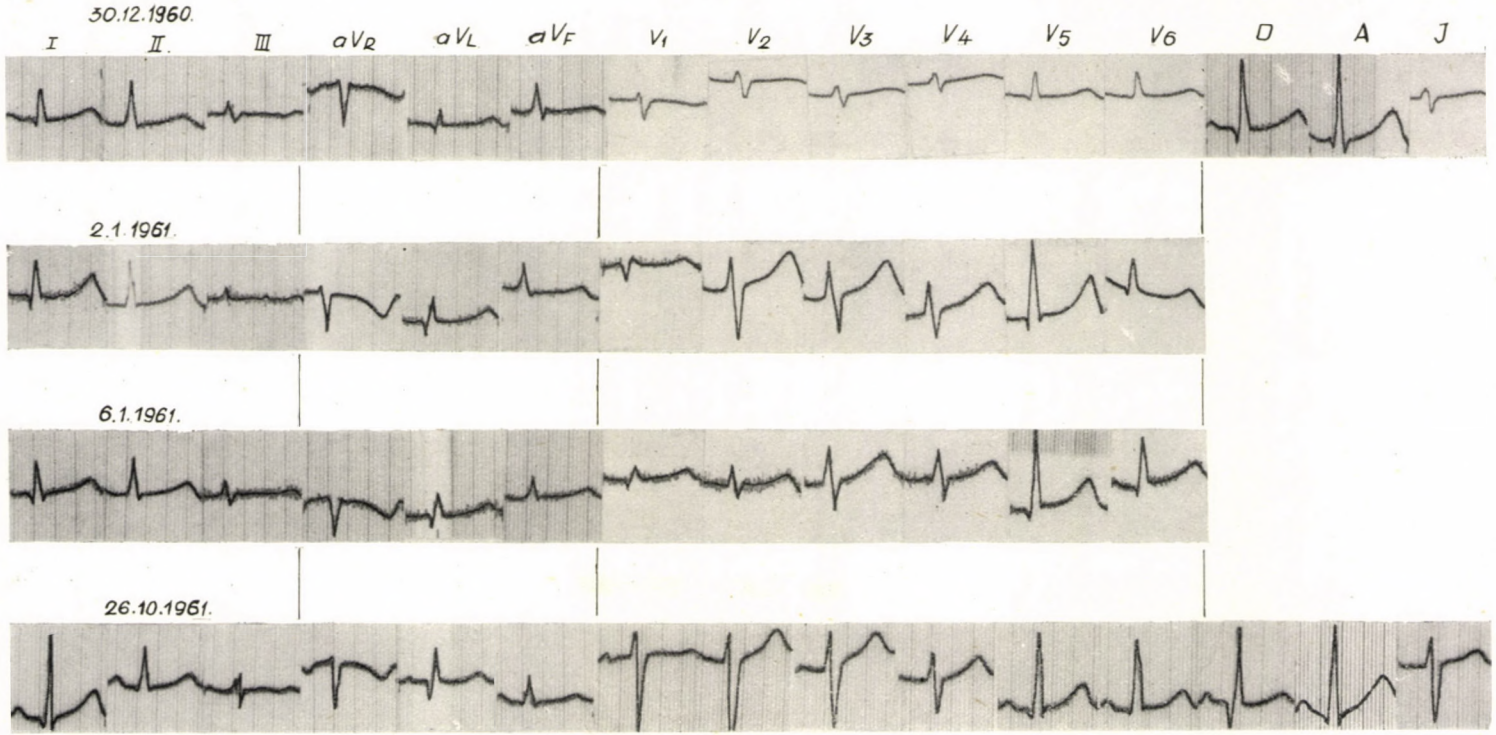

Fig. 3. Permanent deep Q-wave due to herpes zoster

A study of the correlation between the gravity of herpes and the incidence of ECG deviations showed that there were such anomalies in 6 out of 17 mild cases, while among 9 patients suffering from grave, extensive, hemorrhagic herpes (including a case with necroses) 8 showed pathologic ECG tracings. Making a random selection at the Department of Dermatology, among 100 patients we found 7 whose ECG revealed pathologic changes.

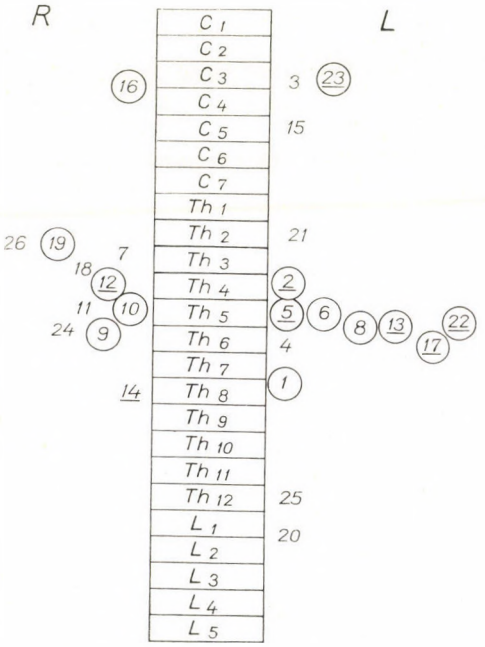

Fig. 4. Interconnections between the site and gravity of herpes zoster and the ECG changes (Number alone means simple herpes zoster; underlined number indicates grave, hemorrhagic cases; encircled number points to ECG changes)

The difference analysed with the χ^2 test was found to be significant statistically.

Fig. 4 presents a general survey of our cases as also the interrelation between the vertebral level of the disease, the affected side, and the ECG deviations. Where the herpes extended over several segments, the number of the cases is indicated in the middle segment (for instance, case No. 17 which covered the segments Th_5, Th_6 and Th_7 is indicated in Th_6); the underlining of the case-number indicates a serious form of the disease (e. g. case No. 12), while a circle around the number means that the ECG showed pathologic changes (e.g. 12). As can be seen from the diagram, in most of our patients the herpes was localized between the segments Th_2 and Th_7. 19 cases

belonged to this category, of which 12 were mild and 7 grave. It was in this group that the incidence of pathologic ECG changes was the highest.

It is now planned to study cases of cervical and lumbar herpes from a cardiologic angle, and compare the results with the present findings. The number of cases does not allow conclusions concerning possible interconnections between the affected side (right or left) and the ECG record.

Except the Q-wave, all ECG changes were transient phenomena, lasting during the eruption period of the herpes. The ECG anomalies were frequently changing, becoming worse or improving, or even disappearing within the space of but 2 to 3 days. Six follow-up examinations, performed after a year, showed — except for the presence of a deep Q-wave in 2 cases — no changes on the ECG, which during the course of the herpes had been abnormal.

Pains in the left side of the thorax with no herpes may be suggestive of an attack of angina pectoris or of cardiac infarct. The correct differential diagnosis is made difficult by the ECG changes associated with the herpes. The pain, however, is lasting and unaccompanied by fever or hypotension. Nor do laboratory tests point to myocardial necrosis. Differentiation is somewhat facilitated by the observation of PASTINSZKY et al., in that, like other viral infections, herpes zoster is also accompanied by hypoprothrombinaemia.

Discussion

There are two theories concerning the pathomechanism of ECG changes due to herpes zoster.

The anomalies may be due to disturbances in cardiac innervation. The sympathetic nerves of the heart take their origin in the segments Th_1 to Th_5. Fibres of the rami communicantes albi run to the stellate ganglion, and it is from the latter that those fibres arise which, as accelerant nerves, run to the cardiac plexus. The antagonistic innervation originates from the nuclei of the bulbar vagus. Herpes zoster covering the segments Th_2 through Th_7 was usually accompanied by a pathologic ECG in our cases, a phenomenon well in harmony with the disturbed sympathetic innervation of the heart due to ganglionitis.

Of recent, the theory of vascular pathogenesis has gained ground. FEYRTER has shown that not only the skin and the nerves but also other organs are attacked by the herpes zoster virus. Involvement of the ganglia is not the rule in cases of herpes zoster, so that the lesion of spinal ganglia need not necessarily be of causal significance. FEYRTER demonstrated hyperergic capillaritis and polyarteriitis-nodosa-like hyperergic arteritis in the vessels of the affected segments (polyarteriitis nodosa zosterica). The arteries of the skin, as well as the nerves, show a symmetrical and metameric arrange-

ment (SIEGLBAUER). It is, according to FEYRTER, through the blood path that the herpes zoster virus gains access to the affected tissues. The ganglia are often but not invariably affected. The vascular genesis seems to be confirmed by cases in which metameric cutaneous manifestations are observed without the involvement of the spinal ganglia or by those which run their course without sensory disturbance, neuralgia or other nervous phenomena (HOCHLEITNER). There are, however, no such proofs of hyperergic capillary changes being associated with herpes zoster as would confirm and morphologically support the theory of vascular origin.

We are indebted to Dr. I. JUVANCZ for the statistical analysis.

REFERENCES

FEYRTER, F.: Hautarzt **5**, 391 (1954).
FEYRTER, F.: Österr. Z. Kinderheilk. **10**, 43 (1954).
HOCHLEITNER, H.: Derm. Wschr. **142**, 1049 (1960).
PASTINSZKY, I., KOVÁCS, E., RÁCZ, I., GESZTI, O.: Derm. Wschr. **132**, 1955 (1955).
SIEGLBAUER, F.: Lehrbuch der normalen Anatomie des Menschen. 8. Aufl. Urban u. Schwarzenberg. München—Berlin, 1958.

István PASTINSZKY, Budapest II. Széher út 78.
István KENEDI, Budapest XIII. Pozsonyi út 14. Hungary.

ÜBER DIE WIRKUNG DES STROPHANTHINS AUF DIE NIERENFUNKTION

Von

F. SOLTI, I. MÁRTON, Judith RÉV und R. HERMANN

1. MEDIZINISCHE KLINIK (DIRECTOR: PROF. DR. I. RUSZNYÁK) DER MEDIZINISCHEN UNIVERSITÄT, BUDAPEST

(Eingegangen am 23. März 1962)

Die Nierenwirkung des Strophanthins wurde bei kreislaufgesunden und bei an chronischer Kreislaufinsuffizienz leidenden Personen und auch in Hundeexperimenten untersucht. Es wurde eine akute bedeutende Erhöhung der Wasser- und Kaliumausscheidung und eine mäßigere der Natriumausscheidung festgestellt. Diese Wirkung wurde sowohl bei Kreislaufgesunden als auch bei an chronischer Kreislaufinsuffizienz leidenden Kranken beobachtet. Die durch Strophanthin verursachte Vermehrung der Wasser- und Salzexkretion beruht vor allem auf einer Herabsetzung der tubulären Resorption und kann nicht mit Veränderungen im Blutkreislauf erklärt werden. Bewertbare Veränderungen des ADH-Serumspiegels können nach Verabfolgung von Strophanthin nicht registriert werden. In Baytinalnarkose, nach Prämedikation mit Dibenzylamin oder nach Durchtrennung des renalen Sympathicus tritt die erwähnte renale Strophanthinwirkung nicht in Erscheinung. Nach Injektion von Strophanthin in den vom Rumpf völlig isolierten Kopfkreislauf kam dieselbe charakteristische Vermehrung der Wasser- und Salzexkretion zustande.

Die Resultate lassen darauf schließen, daß die Wirkung des Strophanthins auf die Nierenfunktion hauptsächlich durch Vermittlung des Zentralnervensystems sowie der zu den Nieren verlaufenden Sympathicusbahnen erfolgt.

Die Wirkung der Digitalisglykoside auf Nierenfunktion, Wasser- und Salzhaushalt wurde oft untersucht. Die Ergebnisse dieser Forschungen und vor allem ihre Bewertung sind nicht einheitlich. In bezug auf die auf den Wasser- und Salzhaushalt ausgeübte Wirkung des Strophanthins (Digitalis) werden drei Erklärungen anerkannt: 1. BODE und GREEF [1] vertreten die Meinung, Digitalis erhöhe den Kaliumspiegel und die Steigerung der renalen K- und Na-Exkretion treten sekundär in Erscheinung. — 2. Nach der Ansicht von FARBER et al. [2] hemmt das Digitalis die ADH-Sekretion; die Wirkung auf den Wasser- und Salzhaushalt sei auf diese Weise zu erklären. — 3. FREY [3] nimmt auf Grund von biochemischen, BRANDES und SUCHOWSKY [4] von histologischen Untersuchungen einen unmittelbaren Einfluß auf die Niere an. — GREMELS [5] sowie HYMAN et al. injizierten Strophanthin in die A. renalis und beobachteten eine prompte Steigerung der Wasser- und Salzausscheidung durch die betreffende Niere. Diese Untersuchungen sprechen also für eine direkte Beeinflussung des Nierenparenchyms durch Digitalis.

In der vorliegenden Mitteilung werden unsere Experimente über die Wirkung des Strophanthins auf die Nierenfunktion erörtert. Im Laufe dieser Experimente waren wir bestrebt zu erforschen, wie sich nach Verabreichung von Strophanthin die Nierenfunktion von Patienten mit intaktem Kreislauf

Tabelle I

Die Nierenwirkung des Strophanthins bei Kreislaufssinsuffizienz

Name: Diagnose:	Alter	Periode:	U	C_K	C_{PAH}	F_f	U_{Na}	E_{Na}	U_K	E_K	RR	P	V_{Dr}	M_V
T. Gy. comb. mitr. vitium	30jährig	VP	1,75	114	380	20	168	2,99	232	3,95	130/80	80	—	4,9
		0—10	—	—	—	—	—	—	—	—	130/80	74	—	4,6
		10—20	3,60	118	445	26,5	222	8,00	160	5,85	120/85	76	—	4,5
		20—30	2,60	77	316	24,0	360	9,35	155	4,03	125/85	74	—	4,4
		30—40	4,20	115	432	26,6	227	9,50	150	6,30	125/80	80	—	5,0
T. K. myodeg. cordis	49jährig	VP	0,78	84	420	22	500	3,90	130	0,82	150/85	76	—	4,5
		0—10	1,40	131	700	19	572	8,0	95	1,33	155/90	78	—	4,6
		10—20	1,70	153	790	19	590	10,0	93	1,58	150/90	72	—	4,2
		20—30	1,20	103	476	22	605	7,30	90	1,08	160/90	74	—	4,6
		30—40	1,60	159	594	27	560	9,00	90	1,44	—	—	—	—
K. L. hypertensio myodeg. cordis	37jährig	VP	1,18	100	460	26	660	8,05	160	2,10	170/105	76	84	3,6
		0—10	1,65	167	600	24	635	10,5	140	2,32	160/110	74	80	3,3
		10—20	2,30	200	800	28	615	14,1	150	3,45	170/110	76	82	3,4
		20—30	2,20	180	785	28	600	13,2	145	3,18	155/100	78	76	3,5
		30—40	1,85	176	595	30	565	10,6	150	3,77	165/100	72	88	3,5
K. S. hypertensio coronariasclerosis	63jährig	VP	1,25	110	365	28	300	3,55	245	3,05	155/100	80	80	2,5
		0—10	1,40	105	362	30	360	5,04	150	2,10	160/95	76	84	2,4
		10—20	1,20	105	460	25	395	4,73	235	2,82	155/95	76	82	2,5
		20—30	1,05	79	320	25	365	3,84	180	1,90	160/95	74	74	2,4
		30—40	1,15	96	386	25	425	4,89	230	2,65	155/95	74	84	2,5
M. O. myodeg. cordis	56jährig	VP	1,18	82	240	34	260	3,12	82	1,00	140/85	80	80	5,0
		0—10	3,08	142	320	36	240	7,40	70	2,15	130/80	72	72	4,6
		10—20	2,20	142	400	—	220	4,85	60	1,32	135/80	74	70	4,7
		20—30	2 00	120	500	24	225	4,50	55	1,10	130/90	76	78	4,6
		30—40	1,37	119	590	40	225	1,83	55	—	135/90	74	76	4,5

B. H. insuff. valv. aortae	30jährig	VP	0,65	55	300	35	210	1,40	90	0,60	140/40	84	105	—
		0—10	1,10	60	320	34	220	2,48	100	1,10	135/35	76	100	—
		10—20	1,05	58	315	35	200	2,10	100	1,05	140/45	72	98	—
		20—30	1,20	66	360	32	220	2,62	90	1,08	130/30	72	102	—
		30—40	1,40	70	405	31	230	3,40	90	1,36	140/35	76	101	—
Sz. S. hypertensio	61jährig	VP	1,20	96	510	26	476	5,70	123	1,48	185/105	74	—	2,9
		0—10	2,40	160	840	18	492	11,8	108	2,60	190/110	74	—	2,9
		10—20	2,30	160	820	18	482	11,1	113	2,60	210/120	64	—	2,7
		20—30	1,90	155	820	22	510	9,7	118	2,24	220/130	60	—	2,6
		30—40	2,00	155	680	27	488	9,75	118	2,36	210/130	74	—	3,5
N. J. comb. mitr. vitium	28jährig	VP	0,80	62	405	15	300	2,40	90	0,72	120/70	84	110	4,9
		0—10	1,50	60	415	15	305	4,70	100	1,50	115/60	76	116	4,6
		10—20	1,40	55	400	15,5	300	4,20	100	1,40	120/60	70	94	4,5
		20—30	1,50	68	460	16	400	6,00	90	1,35	110/55	70	100	4,4
		30—40	2,00	72	500	17	295	5,90	90	1,80	115/60	72	96	4,6
B. J. insuff. valv. bicusp.	40jährig	VP	1,00	70	350	20	250	2,50	120	1,20	140/85	90	115	5,1
		0—10	1,50	74	400	18,5	300	4,50	110	1,65	140/80	82	120	4,7
		10—20	1,40	68	370	19	310	4,55	125	1,75	140/85	74	110	4,6
		20—30	1,55	76	450	17	300	4,70	150	2,30	130/75	80	90	4,6
		30—40	1,50	82	500	17	320	4,80	150	2,25	140/80	92	84	5,2
Á. I. obesitas, myodeg. cardis	48jährig	VP	0,76	103	378	27	485	3,68	135	1,13	140/90	72	—	4,0
		0—10	0,94	132	490	27	495	4,65	135	1,27	150,90	60	—	3,7
		10—20	0,76	107	400	27	530	4,00	150	1,14	160/100	62	—	3,6
		20—30	0,86	115	450	26	530	4,55	175	1,50	160/90	56	—	3,5
		30—40	0,69	80	322	27	490	2,90	180	1,06	160/100	60	—	3,6

Erklärung der Abkürzungen :
Zur Kolumne Periode:
VP = Vorperiode
0—10 = nach Strophanthin die ersten 10 Minuten
10—20 = nach Strophanthin die zweiten 10 Minuten
20—30 = nach Strophanthin die dritten 10 Minuten
30—40 = nach Strophanthin die vierten 10 Minuten

U = Urin ml/Min.
C_{PAH} = PAH clearance
U_{Na} = Urin Natriumkonzentration
U_K = Urin Kaliumkonzentration
P = Pulszahl
K = endogen kreatinin clearance

F_f = Filtrationsfraktion
E_{Na} = Natriumexcretion mg/min
E_K = Kaliumexcretion mg/min
V_{Dr} = Venendruck
M_v = Minutenvolumen, der Starrschen Formel gemäß: 100 + 0,5 × Pulsdruck—0,6 × diastolischer Druck — 0,6 × Alter

Tabelle II

Die Nierenwirkung des Strophanthins bei Kreislaufinsuffizienz

Name: Diagnose:	Alter:	Periode:	U	C_K	C_{PAH}	F_f	U_{Na}	E_{Na}	U_K	E_K	RR	P	V_{Dr}	M_V
		VP	2,00	77	500	13,5	500	6,85	—	—	145/90	76	48	4,3
H. Gy.	39jährig	0—10	3,00	125	1100	11,0	585	17,5	—	—	130/80	82	52	4,2
neurosis		10—20	2,70	94	690	14,0	475	12,8	—	—	130/85	86	50	4,1
		20—30	2,80	97	800	12,0	482	13,5	—	—	130/90	88	46	3,8
		30—40	2,60	95	712	13,0	490	12,7	—	—	130/80	90	44	4,3
		VP	1,80	110	560	21,0	427	7,70	560	10,1	115/70	90	—	5,2
R. J.	34jährig	0—10	4,70	210	680	31,0	325	15,30	262	12,3	120/75	90	—	4,8
neurosis		10—20	3,80	177	530	33,0	328	12,50	265	10,1	120/75	88	—	4,7
		20—30	3,80	187	566	33,0	328	12,5	266	10,1	—	—	—	—
		30—40	4,90	202	740	31,0	327	16,0	267	13,1	110/70	92	—	5,1
		VP	1,50	105	530	20,0	300	4,50	205	3,10	140/80	60	44	3,8
Z. A.	28jährig	0—10	2,00	100	480	19,0	250	5,0	200	4,0	120/65	70	48	4,6
ulcus duodeni		10—20	2,00	98	500	20,0	305	6,10	190	3,80	120/70	72	50	4,8
		20—30	1,50	100	510	20,0	305	4,55	210	3,15	115/65	70	50	4,2
		30—40	1,40	90	460	20,0	300	4,20	220	3,25	120/70	70	44	4,0
		VP	0,42	123	635	19,0	400	1,68	395	1,66	120/80	74	60	5,5
L. J.	60jährig	0—10	0,76	200	583	35,0	440	3,34	415	3,15	110/65	80	60	5,3
gastritis chr.		10—20	0,64	138	520	27,0	405	2,59	365	2,24	110/70	80	66	5,1
		20—30	0,78	153	885	17,0	390	3,04	340	2,65	110/70	82	70	5,1
		30—40	0,42	138	385	36,0	375	1,58	345	1,45	105/65	84	68	5,2

		VP	1,02	104	530	20,0	520	5,31	85	0,87	140/90	60	72	3,9
U. J.	53jährig	0—10	1,80	115	560	21,0	460	8,30	50	9,00	150/95	64	46	3,8
cholelithiasis		10—20	1,40	104	580	18,0	440	6,16	55	7,70	145/95	60	50	3,7
—		20—30	1,25	82	430	19,0	450	5,64	55	6,90	140/90	62	44	4,2
		30—40	1,0	120	580	21,0	525	5,20	70	7,0	135/85	60	42	4,0
		VP	1,10	100	510	20,0	310	3,43	150	1,65	140/80	80	76	4,1
M. N.	50jährig	0—10	2,00	100	620	21,0	300	6,00	150	3,00	140/80	82	68	4,1
colitis		10—20	2,00	90	600	21,0	300	6,00	160	3,20	135/80	74	76	3,7
		20—30	1,50	120	620	20,0	290	4,35	150	1,75	140/80	72	72	3,9
		30—40	1,00	110	560	20,0	325	3,25	160	1,60	140/80	80	76	4,1
		VP	1,40	98	500	20,0	405	5,65	200	2,80	130/80	80	44	4,8
K. L.	40jährig	0—10	1,90	100	530	21,0	400	7,60	210	3,80	130/80	76	40	4,6
neurosis		10—20	2,00	90	540	22,0	410	8,20	210	4,20	—	—	—	—
		20—30	1,70	110	560	20,0	350	6,95	200	3,40	—	—	—	—
		30—40	1,40	110	555	20,0	400	6,40	210	2,90	130/80	80	40	4,8
		VP	2,00	120	600	20,0	350	7,00	150	2,00	120/80	80	36	3,7
A. E.	24jährig	0—10	3,00	116	550	19,0	380	11,4	200	6,00	120/75	76	40	3,7
ulcus ventriculi		10—20	3,00	124	630	20,0	350	10,5	200	6,00	120/75	—	—	—
		20—30	1,80	130	650	20,0	400	7,20	210	5,90	120/75	76	36	3,7
		30—40	1,90	124	600	20,0	400	7,60	200	5,80	120/75	76	40	3,8

Erklärung der Abkürzungen :

Zur Kolumne Periode:
VP = Vorperiode
0—10 = nach Strophanthin die ersten 10 Minuten
10—20 = nach Strophanthin die zweiten 10 Minuten
20—30 = nach Strophanthin die dritten 10 Minuten
30—40 = nach Strophanthin die vierten 10 Minuten

U = Urin ml/Min.
C_{PAH} = PAH clearance
U_{Na} = Urin Natriumkonzentration
U_K = Urin Kaliumkonzentration
P = Pulszahl
K = endogen kreatinin clearance

F_f = Filtrationsfraktion
E_{Na} = Natriumexcretion mg/min.
E_K = Kaliumexcretion mg/min.
V_{Dr} = Venendruck
M_v = Minutenvolumen, der Starrschen Formel gemäß: 100 + 0,5 × Pulsdruck — 0,6 × diastolischer Druck — 0,6 × Alter

bzw. chronischer Kreislaufinsuffizienz gestaltet und wie die Nierenwirkung des Strophanthins zu erklären sei.

Methodik

Die akute Wirkung des Strophanthins auf die Nierenfunktion wurde bei 10 an chronischer Kreislaufinsuffizienz leidenden Patienten, sowie bei 8 Personen mit intaktem Kreislauf beobachtet. Nach drei 10'-igen Urinsammlungs-Vorperioden wurden 0,25 mg Strophanthin K intravenös injiziert und anschließend der Urin in vier 10'-igen Perioden weiter gesammelt. Die Sammlung des Urins erfolgte durch Katheterisieren der Harnblase. Die Nierendurchblutung wurde mittels PAH-Infusion, die Glomerulus-Filtration mittels endogener Kreatinin-Clearance bestimmt. In jeder Periode wurden Blutdruck und Pulsfrequenz gemessen, zumeist auch die Änderungen des venösen Druckes. Die Änderungen des Harnminutenvolumens wurden auf Grund der STARRschen Formen berechnet. (Bezüglich Einzelfragen der experimentellen Methodik verweisen wir auf eine vorhergegangene Mitteilung [7]).

In einer anderen Versuchsreihe untersuchten wir die Rolle des vegetativen Nervensystems in der renalen Wirkung des Strophanthins. Diese Experimente wurden bei 5 Patienten nach Verabreichung von Dibenzylamin (5 mg/kg Körpergewicht in langsamer Tropfinfusion), und bei weiteren 5 Patienten in Baytinalnarkose (langsame i. v. Injektion der 10%igen Lösung bis Patient einschläft) wiederholt. (Die Methodik wurde früher beschrieben [8]).

Die Wirkung des Strophanthins auf die ADH-Sekretion wurde bei 10 dekompensierten sowie 5 kreislaufgesunden Personen untersucht. Die ADH-Bestimmung im Serum erfolgte mit dem modifizierten BURNschen Verfahren [9, 10].

In Tierexperimenten untersuchten wir die neurale Wirkung des Strophanthins auf die Nierenfunktion. Bei 7 Hunden wurde nach Denervation der einen Niere die akute Wirkung des Strophanthins sowohl auf die gesunde wie die denervierte Niere beobachtet. Zwecks Ausschaltung der zur Niere verlaufenden Sympathicusfasern wurde 7 Tage vor dem Experiment, nach Eröffnung im V—VI. Interkostalraum der linksseitige truncus sympathicus bis zum Zwerchfell freigelegt und entfernt [11]. Der Urin wurde mit in die Uretern gebundenen Kanülen aus der gesunden und der denervierten Niere gesondert gesammelt. Die Menge des intravenös verabfolgten Strophanthins betrug 0,33 mg. Die auf die Nierenfunktion ausgeübte Wirkung des in den isolierten Kopfkreislauf injizierten Strophanthins beobachteten wir bei 6 Hundepaaren mit gekreuztem Kreislauf. Das Strophanthin (0,33 mg) wurde langsam in den, vom Rumpfkreislauf isolierten, Kopfkreislauf des Empfängertieres eingespritzt. Die Sammlung des Urins erfolgte durch Ureterkanülen, die durch die Bauchwand herausgeführt wurden. (Einzelheiten s. in unserer diesbezüglichen Mitteilung [12]).

Ergebnisse

Die akute Nierenwirkung des Strophanthins ist in Tabelle I und II dargestellt. Bezüglich der übrigen Experimente werden bloß die Ergebnisse der statistisch-mathematischen Analysen mitgeteilt. In Tabelle I wurden die experimentellen Angaben der an chronischer Kreilaufsinsuffizienz leidenden Patienten, in Tabelle II die der kreislaufgesunden Personen veranschaulicht. Wie ersichtlich, bestehen zwischen den Ergebnissen der beiden Gruppen bloß graduelle Unterschiede. Bezeichnend für die Wirkung des Strophanthins ist, daß die Ausscheidung von Wasser, Kalium und Natrium beträchtlich zunimmt, während sich die glomeruläre Filtration und die PAH-Clearance kaum ändern. Bemerkenswert ist, daß die Strophanthinwirkung auffallend rasch, innerhalb 10 Minuten in Erscheinung trat, Blutdruck, Venendruck, Pulsfrequenz und Minutenvolumen zeigten keine bewertbaren Änderungen.

Somit steht die akute Nierenwirkung des Strophanthins nicht mit der Verbesserung des Kreislaufs in Zusammenhang. Gewisse Unterschiede zwischen den beiden Gruppen zeigten sich in der 4. Periode (30—40 Minuten nach der Injektion). Zu diesem Zeitpunkt nehmen normalerweise Wasser- und Salzausscheidung allmählich ab; bei dekompensierten Herzkranken hält jedoch die Steigerung der strophanthinbedingten Wasser- und Salzexkretion in diesem Zeitpunkt noch an (s. Tabelle III).

Die Ergebnisse der mathematisch-statistischen Analyse der Dibenzylamin und Baytinal-Versuche sind in Tabelle IV veranschaulicht. Die durch Strophanthin verursachte Steigerung der Wasser-, Kalium- und Natriumausscheidung wurde durch beide Pharmaka beträchtlich vermindert.

Der ADH-Serumspiegel blieb nach Strophanthinverabfolgung im allgemeinen unverändert. (Die beobachteten Veränderungen waren nicht signifikant.)

Die mathematisch-statistischen Ergebnisse der Nierendenervationsexperimente sind in Tabelle V dargestellt. Die des größten Teils ihrer sympathischen Innervation beraubte, praktisch als denerviert zu betrachtende Niere zeigte eine ausgesprochen gesteigerte Wasser-, Natrium- und Kaliumexkretion. Auch die glomeruläre Filtration und PAH-Clearance sind in der denervierten Niere gesteigert. Nach Verabfolgung von Strophanthin zeigen die zwei Nieren einen entscheidenden Unterschied, da bei der denervierten Niere die strophanthinbedingte Steigerung der Wasser-, Natrium- und Kaliumausscheidung unterbleibt.

Die durch das Zentralnervensystem übermittelte renale Wirkung des Strophanthins ist in Tabelle VI zusammengefaßt. Wie ersichtlich, erfolgt nach Einspritzung von Strophanthin in den isolierten Kopfkreislauf eine prompte Erhöhung der Wasser-, Natrium- und Kaliumausscheidung, ähnlich wie es bei intakten Tieren beobachtet wurde. Blutdruck, Hirndurchblutung, Pulsfrequenz und Venendruck blieben unverändert. Es muß erwähnt werden, daß nach den mit Evansblau und J^{131} ausgeführten Kontrolluntersuchungen Rumpf- und Kopfkreislauf des Empfängertieres völlig isoliert waren.

Besprechung

Die Nierenwirkung des Strophanthins wurde von mehreren Autoren untersucht, ihre Ergebnisse sind jedoch recht abweichend. Dies dürfte einerseits dadurch zu erklären sein, daß einige Autoren keine Nierenfunktionsprüfung vornahmen bzw. die Untersuchung des Kreislaufs versäumten. Anderseits wurde die Strophanthinwirkung meist in chronischen Experimenten beobachtet, obwohl sich die Wirkung auf die Nierenfunktion rasch entfaltet. In unseren Experimenten erfolgte nach Strophanthingabe eine bedeutende

Tabelle III

Mathematisch-statistische Analyse der akuten Nierenwirkung des Strophanthins
(Änderungen von 20—30 Minuten auf 30—40 Min.)

	Kreislaufgesunde			Kreislaufkranke		
n	8	8	7	10	10	9
$\bar{x}$	—0,06	—0,10	+0,18	+0,02	—0,01	+0,05
s	0,50	1,56	1,32	0,06	0,15	0,07
t	0,36	0,18	0,36	0,89	0,25	1,90
P%	>70	>80	>70	~40,	~80,	~10,
Signifikant	nein	nein	nein	nein	nein	nein

Abkürzungen:
n = Zahl der beobachteten Fälle
$\bar{x}$ = Durchschnittliche Änderung
s = Streuung der Änderungen
t = Studentsche Zahl
P% = Wahrscheinlichkeit

Tabelle IV

Wirkung des Strophanthins auf die Nierenfunktion nach Verabfolgung von Dibenzylamin bzw. in Baytinal-Narkose
(Mathematische Analyse)

	Dibenzylamin			Baytinal		
	U	E_{Na}	E_K	U	E_{Na}	E_K
n	5	5	5	5	5	5
$\bar{x}$	0,14	0,73	0,37	0,18	—1,11	0,30
s	0,32	0,45	0,65	0,35	2,80	0,32
t	1,00	3,65	1,32	1,29	0,89	2,14
P%	>30	< 5	>20	>20	>40	~10
Signifikant	nein	ja	nein	nein	nein	nein

Tabelle V

Strophanthinwirkung auf innervierte und denervierte Niere bei Hunden
(Statistische Analyse. Denerviert — Innerviert)

	U	E_{Na}	E_K
n	7	7	7
$\bar{x}$	—0,08	—0,17	—0,15
s	0,07	0,32	0,13
t	2,90	1,43	3,10
P%	< 5	~20	~ 2
Signifikant	ja	nein	ja

Tabelle VI.

Wirkung des in den isolierten Kopfkreislauf injizierten Strophanthins auf die Nierenfunktion des Hundes
(Statistische Analyse)

	In den Körperkreislauf verabfolgtes Strophanthin. Rechte Niere (linke Niere denerviert)			In den isolierten Kopfkreislauf verabfolgtes Strophanthin		
	V	E_{Na}	E_K	V	E_{Na}	E_K
n	7	7	7	6	6	6
$\bar{x}$	0,19	0,28	0,14	0,34	0,63	0,58
s	0,24	0,24	0,20	0,20	0,33	0,23
t	2,13	2,80	1,40	3,40	4,50	5,80
P% (t)	<10	< 5	~20	<5	<1	<1
P% (binom)	< 5	< 5	< 5			
Signifikant	ja	ja	ja	ja	stark	stark

Zwischen den beiden, durch Strophanthin verursachten Nierenfunktionsänderungen (die eine Dosis wurde in den Rumpfkreislauf verabreicht, wobei das Doppelte der rechten intakten Niere berechnet wurde, die andere Dosis in den isolierten Kopfkreislauf) konnte kein signifikanter Unterschied nachgewiesen werden.

Vergleich der Wirkung des in den Rumpfkreislauf verabfolgten (das Doppelte der rechten intakten Niere berechnet) und in den isolierten Kopfkreislauf injizierten Strophanthins

	V	E_{Na}	E_K
n_1; n_2	6; 7	6; 7	6; 7
diff	—0,04	0,07	0,30
t	0,19	0,30	1,69
P%	>80	>70	>10
Signifikant	nein	nein	nein

Vermehrung der Wasser-, Natrium- und Kaliumausscheidung, in Einklang mit den Ergebnissen von SCHROEDER [14], FARBER et al. [2], WERKÖ et al. [13], GREVE et al. [15] sowie GÖLTNER und SCHWAB [16]. Es konnte auch festgestellt werden, daß die Erhöhung der Wasser- und Salzexkretion hauptsächlich infolge Verminderung der tubulären Wasser- und Salzreabsorption entsteht.

Hinsichtlich der akuten Nierenwirkung des Strophanthins wurde zwischen kreislaufgesunden und an chronischer Kreislaufinsuffizienz leidenden Individuen kein Unterschied verzeichnet, während nach einer halben Stunde bereits Unterschiede in Erscheinung traten: Bei Kreislaufgesunden hörte die Zunahme der Wasser- und Salzausscheidung auf, bei Dekompensierten erhöhte sich

die Natrium- und Kaliumexkretion weiter. Da bei Dekompensierten nach Strophanthingabe nach einiger Zeit (ca. einer halben Stunde) eine Verminderung des zirkulierenden Blutvolumens und eine Erhöhung des Harnminutenvolumens erfolgte, darf angenommen werden, daß die späte Nierenwirkung des Strophanthins infolge Verbesserung des Kreislaufs zustande kommt. Dies dürfte auch eine Erklärung dafür bieten, daß ein Teil der Forscher im chronischen Experiment nach Strophanthingabe nur bei chronischer Kreislaufinsuffizienz eine vermehrte Wasser- und Salzausscheidung beobachten konnte.

Hinsichtlich des Mechanismus der Nierenwirkung des Strophanthins sollen zwei negative Ergebnisse herausgehoben werden:

1. Die auf den Wasser- und Salzhaushalt ausgeübte akute Wirkung des Strophanthins steht mit den Änderungen des Kreislaufs nicht im Zusammenhang. CUGUDDA und AGNISETTA [17] sowie DAVIS et al. [18] erklären die Nierenwirkung des Digitalis mit der Verbesserung des Kreislaufs und der konsekutiven Steigerung der renalen Blutzirkulation. Unsere Experimente widersprechen dieser Annahme, da nach Strophanthingabe die Wasser-, Kalium- und Natriumexkretion ohne bemerkenswerte Veränderungen in Blutkreislauf und Nierendurchblutung akut und bedeutend zunahm. Demnach dürften die erwähnten Faktoren nur bei chronischer Strophanthinwirkung in Betracht gezogen werden.

2. Die Strophanthinwirkung ist nicht der Veränderung der ADH-Sekretion zuzuschreiben. LASCHÉ et al. [19] beobachteten bei Digitalisbehandlung eine Herabsetzung des Serum-ADH-Spiegels. In unseren akuten Experimenten wurde keine Herabsetzung des ADH-Spiegels beobachtet; ein solcher Mechanismus kann also die Wirkung des Strophanthins auf die Nierenfunktion nicht erklären.

Als ein positives Ergebnis unserer Untersuchungen dürfte die Beobachtung bewertet werden, daß das Strophanthin seinen Einfluß auf die Nierenfunktion über das Zentralnervensystem, und hauptsächlich durch Vermittlung des Sympathicus ausübt. Die Baytinal-Experimente bezeugen, daß die akute Nierenwirkung des Strophanthins in der Narkose größtenteils unterbleibt; dies deutet auf die wichtige Rolle des Zentralnervensystems hin. Die Strophanthinwirkung erfolgt in erster Linie durch Vermittlung des Sympathicus, da sowohl die Verabfolgung von Dibenzylamin wie die Denervierung der Niere die Wasser- und Salzausscheidung steigernde akute Strophanthinwirkung hemmen.

Nach den Experimenten von WEINBERG und HALEY [20] sowie unseren eigenen [21] erfolgte die Wirkung des Strophanthins auf die Herzfunktion teils durch Vermittlung des Zentralnervensystems. So lag es an der Hand, einen ähnlichen Mechanismus bezüglich der auf die Nierenfunktion ausgeübten Digitaliswirkung vorauszusetzen. Unsere Hundeversuche mit Strophanthin-

gabe in den isolierten Kopfkreislauf haben diese Annahme bestätigt, da beim Empfängertier die charakteristische Erhöhung der Wasser-, Kalium- und Natriumausscheidung zustande kam. Selbstverständlich schließen unsere Versuche die unmittelbare renale Wirkung des Strophanthins nicht aus, betonen aber die entscheidende Rolle des Zentralnervensystems und der zu den Nieren verlaufenden Sympathicusbahnen.

Für die Ausführung der mathematisch-statistischen Analysen sprechen wir Herren Dr. I. JUVANCZ und J. FISCHER auf diesem Wege unseren Dank aus.

LITERATUR

1. BODE, H., GREEF, K.: Manyn-Schmiedebergs Arch. exp. Path. Pharmakol. **227,** 436 (1956).
2. FARBER, S. J., ALEXANDER, J. D., PELLEGRINO, M. D., EARLE, D. P.: Circulation, 4, 378 (1951).
3. FREY, J.: Clin. chim. Acta (Amsterdam) **1,** 35 (1956).
4. BRANDES, G., SUCHOWSKY, G.: Ärztl. Wschr. **9,** 134 (1954).
5. GREMELS, H.: Naunyn-Schmiedebergs Arch. exp. Path. Pharmakol. **140,** 205 (1929).
6. HYMAN, A. L., JAQUES, W. E., HYMAN, E. S.: Amer. Heart J. **52,** 592 (1956).
7. SOLTI, F., RÉV, J., HERMANN, R., RÉFI, Z.: Magy. belorv. Arch. **13,** 51 (1960).
8. SOLTI, F., RÉV, J., HERMANN, R., PREISICH, P., KOLTAY, E.: Magy. belorv. Arch. **13,** 48 (1960).
9. BURN, J. H.: J. Pharmacol. **49,** 237 (1931).
10. SOLTI, F., RÉV, J.: Kísérl. Orvostud. **99,** 718 (1958).
11. SOLTI, F., PAPP, M., MÁRTON, I.: Magy. belorv. Arch. **13,** 45 (1960).
12. SOLTI, F., FÖLDI, M., MÁRTON, I., TAKÁCS, F.: Magy. belorv. Arch. **13,** 51 (1960).
13. WERKŐ, L., BUCHT, H., EK, J., VARNUSKAS, E.: Cardiologia (Basel) **29,** 305 (1956).
14. SCHROEDER, H. A.: Circulation, **4,** 87 (1951).
15. GREVE, M. J., EDDLEMAN, E. E. JR., WILLIS, K., EISENBERG, S., HARRISON, T. R.: Circulation **3,** 405 (1951).
16. GÖLTNER, R., SCHWAB, M.: Klin. Wschr. **32,** 542 (1954).
17. CUGUDDA, E., AGNISETTA, S.: Fol. cardiol. **11,** 453 (1952).
18. DAVIS, J. O., HOWELL, D. S., HYATT, R. E.: Circulat. Res. **3,** 259 (1955).
19. LASCHÉ, E. M., PERLOTT, W. H., DURANT, T. M.: Amer. J. med. Sci. **222,** 459 (1951).
20. WEINBERG, S. J., HALEY, TH. J.: Circulat. Res. **3,** 103 (1955).
21. SOLTI, F.: Thesis, Budapest, 1957.

Ferenc SOLTI
István MÁRTON
Judith RÉV
Robert HERMANN

Budapest VIII., Korányi S. u. 2/a, I. Belklinika, Ungarn

ANTICOAGULANT THERAPY OF ARTERIAL THROMBOSIS AND EMBOLISM IN THE LIMBS

By

K. BUGÁR-MÉSZÁROS and J. FONÓ

FIRST DEPARTMENT OF MEDICINE, ISTVÁN HOSPITAL, BUDAPEST

(Received May 2, 1962)

The value of treatment with anticoagulant drugs has been studied by comparing the results achieved in 96 cases of arterial thrombosis and embolism in the limbs with those in 62 control cases treated with other drugs. The evidence obtained was greatly in favour of anticoagulant treatment.

Anticoagulant therapy has now been used in this department for over a decade. The results achieved with it in coronary thrombosis [7], thromboembolic cerebral processes [4], phlebothrombosis and thrombophlebitis [6], have been reported earlier.

The present paper is an account of our experience gained with this treatment in arterial thrombosis and embolism in the limbs; it does not deal with the problems of diagnosing such cases, for these have been discussed at length in previous works [1, 2, 3, 5].

In recent years a total of 96 such patients were treated with anticoagulant drugs in this Department: 56 for arterial thrombosis and 40 for embolism. A control group of 62 cases — 22 thrombotic and 40 embolic patients — consisted of cases from the time when anticoagulant therapy had not yet been generally applied in this Department, and partly from patients with conditions contraindicating anticoagulant therapy such as hypertension, haemorrhagic diabetic retinopathy, chronic nephritis, tbc. pulm., etc.

Of the 56 thrombotic patients treated with anticoagulants, 46 were males and 10 females; and of the 40 embolic patients, 17 were males and 23 females. The distribution by sex in the control group was 15 males and 7 females with thrombosis, and 18 males and 22 females with embolism. Taking the two groups together, we find 61 thrombotic males against only 17 females. The explanation is that among our patients thrombosis was in many cases a complication of obliterating arteriosclerosis or endoangiitis (thromboangiitis), conditions far more frequent in men than in women. No such marked difference was noted in the sex distribution of the embolic patients (35 males against 45 females).

The distribution by average age, with the extremes in brackets, was as follows: treated thrombotic patients 52.7 years (17 and 75), their untreated controls 67.3 years (56 and 80); treated embolic cases 58.2 years (39 and 92),

their untreated controls 59.3 years (36 and 80). The difference in the average age of the treated thrombotic patients and their untreated controls finds its explanation in that anticoagulant therapy was contraindicated in a greater proportion of the older than the younger patients; for instance, in those with thrombosis developed as a complication of primary obliterating endoangiitis. As a result, in the treated group there were 11 patients below 40, whereas the age of the youngest member in the control group was 56 years.

Our study includes no patient who was transferred for surgery because better results were to be expected from embolectomy than from drug treatment. Nor does it include seven patients with embolism and one with arterial thrombosis who in addition to anticoagulants had been treated with fibrinolysin. Together with some others who received fibrinolysin, their cases are discussed in a separate report from this Department [8].

Of our 56 patients subjected to anticoagulant treatment for thrombosis, the primary disease was obliterating arteriosclerosis in 39 and endoangiitis in 8, while in the history of 9 patients there was angiospasm due to trauma, frostbite, and excessive smoking. Each of the 22 control patients was suffering from obliterating arteriosclerosis.

Of the 40 patients treated for embolism, 10 had stenosis of the left ostium associated in 5 with absolute arrythmia, 5 suffered from other valvular defects associated in 4 of them with absolute arrythmia, and 23 had arteriosclerotic heart disease accompanied in 15 by absolute arrythmia, and in one patient by a recent cardiac infarct. In two cases the cause of embolism could not be established with certainty.

Of the 40 controls with embolism not treated with anticoagulants 9 had stenosis of the left ostium with absolute arrythmia, another 9 suffered from other valvular defects associated in 8 of them with absolute arrythmia, 21 had arteriosclerotic heart disease accompanied in 8 of them by absolute arrythmia and in one by a cardiac infarct. In one control the cause of embolism could not be established with certainty.

All our 96 patients subjected to anticoagulant treatment were given a coumarin preparation which was either Pelentan [aethylium-di-4-(oxy-coumarinyl) aceticum] or Sintrom [alfa(4'-nitrophenyl)-beta-acetyl-(-4-oxy-coumarin)] or Syncumar [3-(alfa)-4'-nitrophenyl-(beta-acetyl-aethyl)-4-hydroxycoumarin].

During treatment the prothrombin time was kept as far as possible at double the normal. In 36 cases with acute vascular occlusion treatment was started with intravenous or intraarterial injections of heparin. Twenty severe cases were given intraarterial injections of 1 ml (5000 U) daily heparin for from 7 to 29 days while the coumarine therapy was proceeding.

All the patients received in addition vasodilating drugs, such as procaine, tolazoline, papaverine hydrochloride, papaverine nicotinate, acetylcholine

intraarterially; tolazoline, papaverine hydrochloride or nicotinate, ATP, sodium nitrite, bamethane sulphate, intramuscularly; papaverine hydrochloride intravenously; or ethaverina, papaverine hydrochloride, tolazoline, bamethane sulphate orally.

We are of course aware of it that no exact comparison is possible between the anticoagulant-treated and the control groups, partly on account of the substantial difference in the average age of the thrombotic patients in the two groups, and partly because hardly ever are even as few as two cases found that would be equal in severity. Nevertheless, for a truer appreciation of the therapeutical results, there is ground for comparing the levels of the sites of the occlusions in the two groups, and such comparison brings out a certain similarity between the two, as can be seen in Table 1 for our thrombotic patients and in Table 2 for our patients with embolism. Multiple occlusions account for the differences in the total numbers of patients and incidents.

Table 1

Distribution of thrombotic incidents in the limbs by the height of the point of occlusion

	Upper limbs				Lower limbs								Total
	Axillary	Brachial	Radial	Digital	Aorta	Common iliac	External iliac	Femoral	Popliteal	Tibial	Dorsalis pedis	Digital	
Anticoag.-treated	1	3	—	15	—	1	2	11	17	3	3	5	60
Controls	—	—	—	4	2	1	1	7	11	—	—	1	27

Table 2

Distribution of embolic incidents in the limbs by the height of the point of occlusion

	Upper Limbs				Lower limbs								Total
	Axillary	Brachial	Radial	Digital	Aorta	Common iliac	External iliac	Femoral	Popliteal	Tibial	Dorsalis pedis	Digital	
Anticoag.-treated	1	4	2	—	1	—	2	9	23	5	—	1	48
Controls	—	4	—	—	—	—	1	17	21	3	—	—	46

The outcome of the cases is summarized in Table 3. Individual patients were classified as markedly improved when they had ceased to have pains at rest, recovered from pregangrene, showed a rise in the oscillometric index,

or regained, at least to a considerable extent, their ability to walk. As slightly improved were regarded the patients in whom no or hardly any rise in the oscillometric index and only moderate improvement in walking but considerable relief of pain were seen, or in whom moist gangrene was turned into the dry type. The patient's condition was considered deteriorated when progressing thrombosis, or thrombosis associated with embolism had aggravated the vascular obstruction, respectively when the pregangrenous state had developed into gangrene, with or without the necessity of amputation. Deaths also came under this heading.

Table 3

Summarized results of cases studied

		Impaired		Unchanged	Deteriorated	Together	Increase of oscillometric index, per cent
		markedly	slightly				
Thrombosis	Anticoag.-treated	40	7	6	3	56	157
	Controls	2	8	2	10	22	33
Embolism	Anticoag.-treated	30	6	1	3	40	540
	Controls	11	20	1	8	40	152

Table 3 shows that better results were obtained in the anticoagulant treated group than in the controls.

The percentage rise in the oscillometric index refers to measurements made in the lower part of the affected lower limbs, respectively in the forearm of the affected upper extremities.

Table 3 may provoke objection on the ground that whereas obliterating arteriosclerosis was the primary disease in all the thrombotic controls, it was not the primary disease in 17 of the thrombotic patients treated with anticoagulants. To meet this objection, Table 4 separately presents the results of anticoagulant treatment in those 39 patients who had obliterating arteriosclerosis associated with thrombosis of the limb. Their average age was 58.8 years. Comparison with the control data which are appearing also in Table 3, clearly shows the favourable results obtained with anticoagulants.

In evaluating our results, a point of importance is that the great majority of our patients with arterial thrombosis were first seen relatively late after onset: only 4 cases in each the treated and the control group were examined within 24 hours.

Of the 40 cases treated with anticoagulants for embolism 18 patients only, and of the 40 controls 21 patients, were seen within 24 hours after onset;

Table 4

Therapeutic results in obliterating arteriosclerosis associated with limb-thrombosis

	Impaired		Unchanged	Deteriorated	Together	Increase of oscillometric index, per cent
	markedly	slightly				
Anticoag.-treated	25	6	5	3	39	124
Controls	2	8	2	10	22	33

the rest in 2 to 45 days. The therapeutical results achieved in the embolic patients admitted 24 hours of onset are summarized in Table 5. They clearly

Table 5

Therapeutic results in limb embolism first seen within 24 hours after onset

	Impaired		Deteriorated	Together	Increase of oscillometric index, per cent
	markedly	slightly			
Anticoag.-treated	17	—	1	18	639
Controls	6	9	6	21	248

establish the value of anticoagulants in the treatment of limb embolism: in the treated group of 18 patients as many as 17 showed marked improvement; in the control group of 21, not more than 6. In the control group as many as 6 cases had deteriorated so that amputation was necessary, whereas in the treated group there was only one case which became worse, and even in that case no gangrene but lethal cardiac decompensation ensued on the 15th day.

The therapeutical result was better in our than in HAIMOVICI's cases with embolism: 4 of his patients died an early death, and 2 of the remaining 12 developed gangrene. JACOBS, in his monograph has failed to separate the results he obtained with anticoagulants in a group of 30 patients with limb embolism, from those he achieved in another group by the use of vasodilator drugs. What his added results do reveal, however, is that the limb could be saved in not more than 60 per cent in his medically treated cases of embolic occlusion that had been diagnosed within 10 hours of onset.

In the pregangrenous category, too, there was a marked difference between the therapeutic results achieved with anticoagulant drugs and the controls. On the evidence of Table 6, 18 out of 20 patients showed improvement in the anticoagulant-treated group, but only 2 out of 10 in the control group. In the gangrenous category for which the details are presented in the same

Table 6

Therapeutic results in pregangrenous and gangrenous patients

		Impaired	Unchanged	Deteriorated	Total
Pregangrene	anticoag.-treated	18	1	1	20
	controls	2	1	7	10
Gangrene	anticoag.-treated	5	5	4	14
	controls	4	—	6	10

table, this difference is less conspicuous. Still, the superiority of the anticoagulant drugs is clearly shown by the fact that only 4 deteriorated among the 14 patients so treated, against 6 failures among the 10 controls.

Another fact in favour of the anticoagulants is that amputation had to be resorted to in 4 out of 96 treated patients (4.2 per cent) but in as many as 8 of 62 controls (12.8 per cent). Twenty patients with extremely severe circulatory disturbances — 8 embolic and 12 thrombotic arterial occlusions — were treated with 1 ml (5000 U) heparin daily, by the intraarterial route. This produced marked improvement in 14, slight improvement in 1, and none in 5 patients. In 2 of the latter, amputation became unavoidable: in one for popliteal embolism, in the other for popliteal thrombosis.

A group of 16 patients needs still to be mentioned, in whom thrombosis was noted in the digital arteries only. Anticoagulant treatment produced marked improvement in all of them, with relief of cyanosis and pain. In 5 cases the toes and in 11 the fingers were affected.

It seems worth while to describe one of these cases.

D. L., a factory workman, 74 years old, had noticed about 3 weeks prior to admission, numbness in the left index finger followed by coldness, pain and swelling. At admission the finger was cold, the ungual phalanx cyanosed and sensitive to pressure. As a sign of arterial obliteration and constriction, the rheoangiogram showed marked deformation with a low curve showing flat peaks and complete absence of dicrotism (Fig. 1). Oscillometry was normal in both forearms: 4.5 Pachon units on the right, and 5.0 on the left side. Pelentan was administered for 11 days, with 3 tablets of papaverine nicotinate daily and injections of sodium nitrite. The condition gradually improved, pain and cyanosis subsided, and the finger got warm. A second rheoangiogram showed a higher curve with pointed peaks and dicrotism (Fig. 2). The rheoangiogram and the electrocardiogram of lead II seen in Figs. 1 and 2 were recorded synchronously.

No complication was noted to occur in the course of anticoagulant therapy except in a single case in the form of a subcutaneous haematoma.

PERLICK, and also MARX and HASSE, hold it inadvisable to administer intraarterial injections to patients who are under anticoagulant treatment.

We cannot subscribe to this view, for we are regularly administering heparin intraarterially and see no complications if the patients follow the instruction to keep a piece of cottonwool pressed firmly to the site of the injection for 15 minutes. We do, on the other hand, agree with the said authors in what

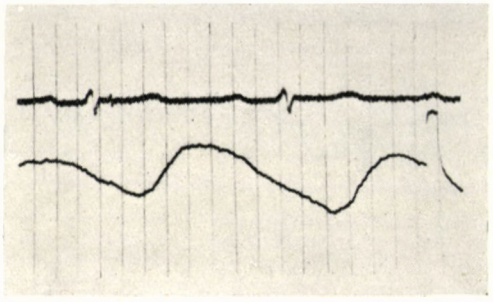

Fig. 1

Cole and Kleitsch, and also Owens and Smith, have likewise emphasized, namely, that procaine block of the lumbar sympathetic ganglions is contraindicated because it may give rise to retroperitoneal haematoma. Therefore, whenever in a fresh case of embolism procaine blockage is found to be unavoidable, we always inject the drug before we begin the treatment with an anticoagulant agent. From this principle we have so far deviated in a single

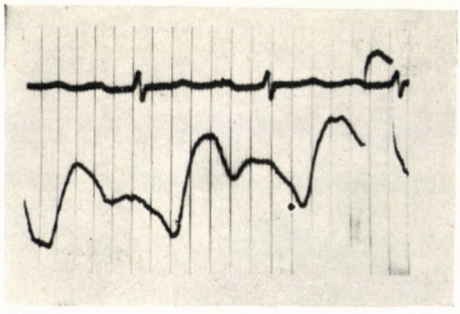

Fig. 2

case; in this it was found that an embolic lower limb of a patient with stenosis of the left ostium, who because of her cardiac condition would not have survived amputation, could not be saved except perhaps by a block. Fortunately, we succeeded in saving the limb, free from any haemorrhagic complications. Maintained on long-term anticoagulant therapy, the patient then lived for two and a half years without recurrence of embolic episodes. Her death was due to cardiac decompensation.

On the basis of our experience we recommend anticoagulant treatment in all cases of arterial thrombotic occlusion in the limbs, provided this is not contraindicated. In the treatment of embolic occlusions, embolectomy is the method of choice, if conditions are favourable. If not, some anticoagulant drug should be prescribed. This is also indicated after embolectomy to prevent fresh embolic incidents. Fibrinolysin dissolving thrombi and emboli has been an advance in the medical treatment of these conditions, but because it is unable to prevent the formation of new thrombi, it must be combined with anticoagulant drugs. This combined treatment is apparently more efficaceous than are anticoagulant drugs in themselves. Therefore, if we have opportunity to make such combined treatment, we prefer it in cases of recent thromboembolism.

Following the practice of WRIGHT, OWREN, WOOD and CONN, and MCCOOK et al., this Department has now applied long-term anticoagulant therapy for about 3 years with the aim of preventing embolism. Such preventive treatment is administered chiefly to patients who have had embolism due to heart disease or arteriosclerosis and who have recovered from coronary or cerebral thrombosis or recurrent phlebothrombosis. The promising results will be reported elsewhere.

REFERENCES

1. BUGÁR-MÉSZÁROS, K.: Orv. Hetil. **93,** 405 (1952); Z. ges. Inn. Med. **9,** 751 (1954).
2. BUGÁR-MÉSZÁROS, K.: Perifériás érbetegségek. Művelt Nép, Budapest, 1955.
3. BUGÁR-MÉSZÁROS, K.: Peripheriás keringési zavarok. Medicina, Budapest, 1961.
4. BUGÁR-MÉSZÁROS, K., KOVÁCS, F., FONÓ, J.: Acta med. Acad. Sci. hung. **16,** 329 (1960).
5. BUGÁR-MÉSZÁROS, K., OKOS, G.: Orv. Hetil. **93,** 664 (1952).
6. BUGÁR-MÉSZÁROS, K., OKOS, G.: Acta med. Acad. Sci. hung. **16,** 313 (1960).
7. BUGÁR-MÉSZÁROS, K., PRAZNOVSZKY, M.: Acta med. Acad. Sci. hung. **16,** 237 (1960).
8. BUGÁR-MÉSZÁROS, K., CSERVENY, M.: Acta med. Acad. Sci. hung. **18,** 335 (1962).
9. COLE, F., KLEITSCH, W. P.: J. Amer. med. Ass. **147,** 1233 (1951).
10. DEMBOWSKI, V.: Akute arterielle Gefäßverschlüsse. In Angiologie ed. M. Ratschow. Thieme, Stuttgart, 1959.
11. HAIMOVICI, H.: Angiology **1,** 20 (1950).
12. JACOBS, A. L.: Arterial Embolism in the Limbs. Livingstone, Edinburgh & London 1959.
13. MARX, H., HASSE, H. M.: Dtsch. med. Wschr. **85,** 355 (1961).
14. MCCOOK, J., UGUET, E., LLANES, P., BRUNET, P., CHARLES, D., BEAUBALLET, P., CRESPO, L.: Angiology **10,** 165 (1959).
15. OWENS, J. C., SMITH, A. J.: Angiology **4,** 23 (1953).
16. OWREN, P. A.: Proc. 5th European Congress of Haematology **1955,** 110; Northw. Med. **56,** 258 (1957); Excerpta med. Sect. XVIII, **2,** 320 (1958).
17. PERLICK, E.: Antikoagulantien. II. ed. Thieme, Leipzig 1959.
18. WOOD, J. C., CONN, H. L.: Circulation **10,** 517 (1954).
19. WRIGHT, I. S.: J. Amer. med. Ass. **163,** 918 (1957).

Károly BUGÁR-MÉSZÁROS
József FONÓ
Budapest IX., Nagyvárad tér I. István kórház, Hungary

ÜBER DIE WIRKUNG DES CHLORPROPAMID AUF DIE GLYKONEOGENESE DER LEBER

Von

A. KÁLDOR und G. POGÁTSA

II. MEDIZINISCHE KLINIK (DIREKTOR: PROF. DR. P. GÖMÖRI) DER MEDIZINISCHEN UNIVERSITÄT

(Eingegangen am 7. Mai 1962)

Es wurde beobachtet, daß die auf Alanin- und Brenztraubensäure-Wirkung im Perfusionspräparat zustandekommende verminderte Zuckerabgabe der Leber durch Chlorpropamid verhindert werden kann, mit Glykokol jedoch kann eine ähnliche Wirkung nicht beobachtet werden. Wird Brenztraubensäure verabreicht, so beeinflußt Chlorpropamid auch die Senkung des Glykogenspiegels der Leber nicht.

Die Wirkung der oralen Antidiabetika auf die Glykoneogenese ist im allgemeinen nicht bekannt. Obwohl durch Hemmung der Nebennierenrindenfunktion die Glykoneogenese der Leber in der Regel herabgesetzt werden kann, sind die bei der peroralen Diabetesbehandlung angewandten Sulfonylureaderivate in den üblichen Dosen ohne Wirkung auf die Nebennierenrindenfunktion, und die hypoglykämische Wirkung entsteht offenbar nicht durch Hemmung der Nebennierenrinde [1]

Über die auf die Glykoneogenese ausgeübte Wirkung der erwähnten Mittel geben auch andere Untersuchungen keine Aufklärung. Das Stickstoffgleichgewicht der mit Sulfonylureapräparaten erfolgreich behandelten Patienten bleibt unverändert [2], es ist sogar verminderte tägliche Stickstoff-Ausscheidung festzustellen [3]. BORNSTEIN wies darauf hin, daß Carbutamid und Tolbutamid die Alanin-Transaminase hemmen, was eher auf verminderte Glykogenese hinweist [4].

Zahlreiche Experimente beweisen unmittelbare Leberwirkung der peroralen Antidiabetika. Es wird sogar angenommen, daß im Zustandekommen der akuten hypoglykämischen Wirkung die verminderte Zuckerabgabe der Leber eine entscheidende Rolle spielt [5]. Unsere vorangegangenen Experimente bewiesen, daß die Zuckerabgabe der Rattenleber im Perfusionspräparat unter Wirkung verschiedener hypoglykämischer Sulfonylureapräparaten abnimmt, wenn aber Sulfonylureapräparate ohne hypoglykämische Wirkung, oder die in der peroralen Diabetesbehandlung ebenfalls angewandte Biguanidderivate mit abweichendem Wirkungsmechanismus verabfolgt werden, so bleibt diese Wirkung aus [6, 7].

Es ergibt sich die Frage, auf welche Weise der Eiweißstoffwechsel der isolierten Leber mit Sulfonylureapräparaten zu beeinflussen ist. Experimentelle Untersuchungen wurden durchgeführt, um die Veränderungen der Zucker-

abgabe der Rattenleber zu registrieren, falls die Infusionsflüssigkeit auch Aminosäuren enthält. Am geeignetsten schien die Anwendung von Alanin, da diese Aminosäure sich bei der Transaminierung zuerst in Brenztraubensäure, des weiteren durch den Trikarboxylsäurezyklus in Kohlensäure umwandelt. Werden andere Aminosäuren angewandt, so muß mit anderen Stoffwechselvorgängen gerechnet werden. Um die weiteren Kettenglieder des Alaninabbaus ebenfalls untersuchen zu können, schien es wichtig, außer Alanin auch die Brenztraubensäure zu prüfen. Im Laufe der Experimente wurde von den übrigen Aminosäuren Glykokoll angewendet.

Methodik

An 120—160 g schweren, aus derselben Zucht stammenden, mit Standarddiät ernährten Albinoratten beiden Geschlechts wurde die Issekutzsche isolierte Leberperfusion eingestellt. Durch eine, in die V. portae eingelegte Kanüle wurde mit glukosefreier Tyrode-Lösung nach 20minutiger Vorperiode, bei konstanter Temperatur von 37° C, unter Sicherung einer gleichmäßigen Oxygenisation, mit 5 ml/min Geschwindigkeit perfundiert. In halbstündigen Zeitabschnitten wurden aus der Perfusionsflüßigkeit mit der Hagedorn—Jensenschen Methode Blutzuckerbestimmungen durchgeführt. Der glukosefreien Tyrode-Lösung wurde einerseits 100 mg% Alanin, anderseits die gleiche Menge Brenztraubensäure bzw. Glykokoll zugeführt. In weiteren Experimenten erfolgte die Zugabe von 100 mg% Chlorpropamid (Chlorphenylsulphonyl-propylcarbamid), sodann identische Dosen von Chlorpropamid mit einer der erwähnten Aminosäuren zusammen. Als Kontrolle diente die Zuckerabgabe der mit glukosefreier Tyrode-Lösung durchströmten Leber. In den Abbildungen sind die Resultate in mg Glukose pro g Leber pro Stunde-Werten angegeben. Eine Gruppe enthält die bei Durchströmung von 7—12 Rattenlebern gewonnenen Werte; »n« bedeutet die Zahl der Fälle, die Streuung ist in den entsprechenden Kolumnen sichtbar. In einem Teil der Experimente wurde der Glykogengehalt der Leber zu Beginn und am Ende der Perfusion mit der Good—Kramer—Somogyischen Methode bestimmt.

Resultate

Abb. 1 zeigt die mit Alanin durchgeführten Experimente. In der weißen Kolumne sind die Werte der Kontrollgruppe, in der schief schraffierten die der Alanin-Gruppe dargestellt, die quadratisch schraffierte zeigt die Resultate der Alanin- und Chlorpropamid-Gruppe und die schwarze die der mit Chlorpropamid behandelten Tiere. Auf Alaninwirkung nahm die Glukoseabgabe in der 1. und 2. Stunde signifikant ab. Auch bei Chlorpropamid war signifikante Verminderung zu verzeichnen. Wurden Alanin und Chlorpropamid gemeinsam angewendet, so konnte im Vergleich zur Kontrolle keine signifikante Verminderung in der Glukoseabgabe der Leber festgestellt werden. In der 1. Stunde war die Differenz zwischen den Alanin bzw. Alanin und Chlorpropamid enthaltenden Gruppen ebenfalls signifikant.

Abb. 2 enthält die Resultate der mit Brenztraubensäure durchgeführten Experimente. Die Werte der Kontrollgruppe sind ebenfalls in der weißen Rubrik wiedergegeben, die schiefe Schraffierung entspricht den Brenztraubensäure-Versuchen, die quadratisch schraffierte den mit Brenz-

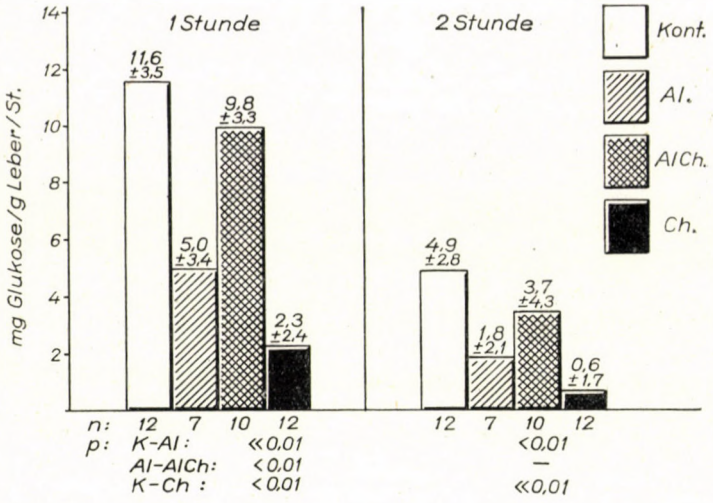

Abb. 1

traubensäure und Chlorpropamid-Lösung enthaltenen Werten. Brenztraubensäure bewirkte in der 1. Stunde eine signifikante Abnahme der Zuckerabgabe und obwohl die gemeinsame Anwendung von Brenztraubensäure und Chlorpropamid in der 1. Stunde ebenfalls zu signifikanter Verminderung führte, war in der Glukoseabgabe in der 2. Stunde kein wesentlicher Unterschied im Vergleich zur Kontrolle nachzuweisen. Werden die Werte der nur Brenztraubensäure enthaltenden Gruppe mit der Brenztraubensäure und Chlorpropamid enthaltenden Gruppe verglichen, so war der Unterschied in der 2.

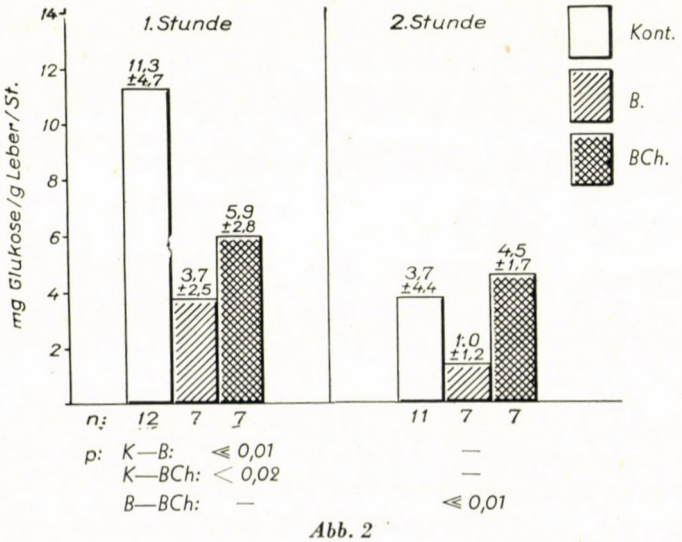

Abb. 2

Stunde — ähnlich wie im Fall der Alanin-Gruppe — signifikant. Die Resultate der Glykogenbestimmungen sind in Tab. I dargestellt. Bei der Kontrollgruppe und bei der Brenztraubensäure enthaltenden Gruppe war die Senkung des Leberglykogenspiegels vom Beginn bis zum Ende der Perfusion signifikant, bei der Brenztraubensäure und Chlorpropamid enthaltenden Gruppe konnte dagegen keine Signifikanz nachgewiesen werden.

In Abb. 3 sind die Resultate der mit Glykokoll durchgeführten Experimente sichtbar. Die weiße Kolumne zeigt die Werte der Kontrollgruppe,

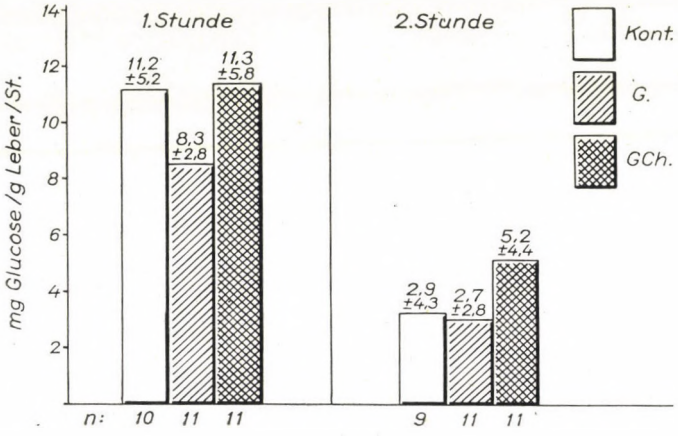

Abb. 3

die schief schraffierte die mit Glykokoll gewonnenen Resultate, und in der quadratisch schraffierten Kolumne sind die bei der gemeinsamen Anwendung von Glykokoll und Chlorpropamid erzielten Ergebnisse registriert. Auf Glykokollwirkung wurde in der Zuckerabgabe der Leber, weder im Vergleich zur Kontrollgruppe, noch zur Glykokoll und Chlorpropamid enthaltenden Gruppe, keine signifikante Veränderung verzeichnet. Die Glykogenresultate dieser Serie sind in Tab. II zusammengestellt. Die signifikante Abnahme des Leberglykogenspiegels am Ende der Perfusion erfolgte in sämtlichen Fällen.

Besprechung

Der in der Leber verlaufende Aminosäurenstoffwechsel ist nicht in allen Einzelheiten geklärt. Die in die Leber gelangenden Aminosäuren umwandeln sich im allgemeinen in Glukose-6-Phosphat und in Ketonsäuren, dadurch wird die Aktivität der Glukose-6-Phosphatase gesteigert und dies

Tabelle I

Veränderung des Leberglykogengehaltes nach Durchströmung von zuckerfreier Tyrodelösung (g Glukose/100 g Leber)

Kontrollgruppe				100 mg% Brenztraubensäure				100 mg% Brenztraubensäure und 100 mg% Chlorpropamid			
v. B.	n. B.	Diff.	%	v. B.	n. B.	Diff.	%	v. B.	n. B.	Diff.	%
1,41	0,67	0,74	—52	1,31	0,52	0,79	—60	0,98	0,62	0,36	—37
2,68	0,84	1,84	—69	0,38	0,20	0,18	—48	1,04	0,75	0,29	—28
4,23	0,56	3,67	—87	4,44	2,94	1,50	—34	0,61	0,53	0,08	—13
1,17	0,31	0,86	—74	0,56	0,30	0,26	—47	0,93	0,74	0,19	—20
1,38	0,18	1,20	—87	1,54	1,30	0,24	—16	1,52	0,63	0,89	—59
2,52	0,37	2,15	—85	4,20	1,46	2,74	—65	7,33	1,70	5,63	—77
4,34	2,17	2,17	—50	1,71	1,08	0,63	—37	2,30	3,44	1,14	+52
4,67	0,97	3,70	—79								
2,95	0,19	2,76	—94								
3,87	0,85	3,02	—78								
2,56	0,78	1,78	—70								
4,02	2,40	1,62	—40								
$\bar{x}$: 2,98	0,86	2,12	—72	2,02	1,10	0,92	—44	2,10	1,20	0,90	—43
s: ±1,24	±0,71	±1,00	±17	±1,65	±1,04	±0,93	±21	±2,73	±1,06	±2,18	±41
n: 12	12	12	12	7	7	7	7	7	7	7	7
S:		11				6				6	
t:		7,3103				2,6286				1,1538	
p:		≪0,01				<0,02				<0,50	

Tabelle II

Veränderung des Leberglykogengehaltes nach Durchströmung von zuckerfreier Tyrodelösung (g Glukose/100 g Leber)

Kontrollgruppe				100 mg% Glykokoll				100 mg% Glykokoll und 100 mg% Chlorpropamid			
v. B.	n. B.	Diff.	%	v. B.	n. B.	Diff.	%	v. B.	n. B.	Diff.	%
1,41	0,67	0,74	—52	1,17	0,83	0,34	—29	1,15	0,62	0,53	—46
2,68	0,84	1,84	—69	2,62	1,56	1,06	—39	1,72	0,24	1,48	—88
4,23	0,56	3,67	—87	4,72	1,51	3,21	—68	1,11	0,66	0,45	—94
1,17	0,31	0,86	—74	3,58	3,52	0,07	— 2	5,32	1,74	3,57	—68
1,38	0,18	1,20	—87	2,24	0,46	1,78	—78	4,51	1,79	2,72	—60
2,52	0,37	2,15	—85	5,40	1,33	4,07	—76	3,46	1,72	1,74	—52
4,34	2,17	2,17	—50	3,79	1,09	2,70	—71	4,51	1,89	2,62	—58
4,67	0,97	3,70	—79	1,95	0,61	1,34	—72	2,86	0,67	2,19	—76
2,95	0,19	2,76	—94	3,91	1,86	2,05	—52	4,69	0,39	4,39	—92
3,87	0,85	3,02	—78	2,38	0,59	1,79	—75	3,30	0,68	2,62	—79
x: 2,92	0,71	2,21	—76	3,18	1,34	1,94	—56	3,26	1,04	2,22	—71
s: ±1,32	±0,59	±1,08	±23	±1,33	±0,90	±1,06	±28	±1,53	±0,66	±1,23	±17
n: 10	10	10	10	10	10	10	10	10	10	10	10
S:		9				9				9	
t:		6,5000				6,0606				6,6154	
p:		≪0,01				≪0,01				≪0,01	

bewirkt eine erhöhte Zuckerabgabe der Leber [8]. Anderseits ist es auch bekannt, daß bei Ratten große Dosen bestimmter Aminosäuren tödliche Hypoglykämie verursachen können. Andere Aminosäuren verhindern dagegen das Zustandekommen dieser hypoglykämischen Wirkung [9]. Durch Aminosäureperfusion wurde in der Rattenleber eine Zunahme der Eiweißsynthese erzielt [10]. Die beschriebenen Experimente sprechen dafür, daß die Aminosäuren die Zuckerabgabe der Rattenleber verschiedenartig beeinflussen. Während auf Alaninwirkung die Zuckerabgabe sich im allgemeinen vermindert, kann nach Glykokollverabreichung diese Wirkung nicht registriert werden. Die mit Brenztraubensäure hervorgerufene verminderte Zuckerabgabe weist dagegen darauf hin, daß der beobachtete Effekt nicht bei der Umwandlung des Alanins in Brenztraubensäure, sondern später zustandekommt. Die vermindernde Wirkung des Alanins bzw. der Brenztraubensäure auf die Zuckerabgabe kann mit Chlorpropamid aufgehoben werden. Glykokoll übte keine solche Wirkung aus. Gleichzeitig fanden wir, daß die bei der Glykokollperfusion zustandekommende Verminderung des Leberglykogens durch Chlorpropamid gehemmt werden kann, während die durch Brenztraubensäure hervorgerufene Glykogenspiegelsenkung durch Chlorpropamid nicht beeinflußt wird. Aus den Resultaten geht hervor, daß die in der Leber verlaufende Glykoneogenese in bestimmten Fällen durch hypoglykämisierende Sulfonylureaderivate beeinflußt werden kann; der Mechanismus des Vorganges soll durch weitere Untersuchungen geklärt werden.

LITERATUR

1. Pfeiffer, E. F., Schöffling, K., Steigerwald, H.: Dtsch. med. Wschr. **81,** 838 (1956).
2. Fajans, S. S., Louis, L. H., Selter, H. S., Johnson, R. D., Gittler, R. D., Hennes, A. R., Wajchenberg, B. L., Ackermann, I. P., Conn, J. W.: Metabolism **5,** 820 (1956).
3. Duncan, L. J. P., Baird, J. D., Dunlop, D. M.: Brit. med. J. **2,** 433 (1956).
4. Bornstein, J.: Nature (Lond.) **179,** 534 (1957).
5. Ashmore, J., Cahill, G. F., Earle, A. S., Zottu, S.: Diabetes **7,** 1 (1958).
6. Káldor, A., Pogátsa, G.: IV. Intern. Kong. für Diabetes, Genf, 1961.
7. Káldor, A., Pogátsa, G.: Orv. Hetil. **103,** 1985 (1962).
8. Levine, R.: Amer. J. Med. **31,** 901 (1961).
9. Greenstein, J. P.: Arch. Biochem. **58,** 253 (1955).
10. Miller, L. L., Bly, G. G., Watson, M. L., Bale, F. W.: J. exp. Med. **94,** 431 (1951).

Antal Káldor, Gábor Pogátsa | Budapest VIII., Szentkirályi u. 46. II. Belklinika, Ungarn

THE EFFECT OF COCARBOXYLASE ON CARDIAC OUTPUT IN ACUTE HYPOXIA

By

Z. NAGY and J. SKOLNIK

SECOND DEPARTMENT OF MEDICINE (DIRECTOR PROF. P. GÖMÖRI), UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received May 9, 1962)

In the dog, the increase of cardiac output induced by acute arterial hypoxia has been found to be reduced by cocarboxylase in spite of the fact that there was no change in the degree of arterial desaturation. Under normal oxygen saturation TPP had no effect on cardiac output.

Acute arterial hypoxia is generally referred to in literature as one of the pathophysiologic conditions of which an increase of cardiac output is characteristic — as in the case of anaemia, histotoxic hypoxia, beri-beri, hyperthyroidism or arterio-venous fistula. Many common features are to be found in all of them, such as an acceleration of circulation and a decrease of the arterio-venous oxygen difference in addition to the augmented cardiac output. The rise in cardiac output ensures adequate oxygen supply for the tissues despite reduced arterial oxygen saturation. In chronic hypoxic states (e. g. congenital valvular heart failure, pulmonary emphysema, or a longer stay at higher altitudes) polyglobulia, the increase in oxygen transporting capacity compensates the deficit [1—12]. Although the increase of cardiac output in acute arterial hypoxia is widely known, knowledge is scarce as to the mechanism responsible for the increase. There is no evidence available as to the role played by the central nervous system in this rise since no alteration in cardiac output has been detected following bilateral carotid artery occlusion [13], nor has there been any change revealed by experiments where isolated head hypoxia was established; whereas in isolated trunk hypoxia — with the head supplied by normally saturated blood — an increase in cardiac output occurred [5]. The alteration in tissue metabolism may presumably account for the increase through the liberated and possibly accumulated metabolites having a direct vasodilating effect [14]. The same mechanism might lead to an increase of cardiac output as in thiamine deficiency. In OCHOA's view [15], the biologically active thiamine-pyrophosphate (TPP or cocarboxylase) loses its effect under hypoxic conditions. It seems that the inactivation of the cocarboxylase system may induce some metabolic change in hypoxaemia and this would account for the increase of cardiac output. We have, therefore, investigated the influence of cocarboxylase

(Berolase, Hoffmann-La Roche) administered intravenously on the increase of cardiac output caused by acute arterial hypoxia.

Methods

The experiments were made on 35 dogs of both sexes weighing 10—20 kg, anaesthesized with chloralose (0.10 g/kg body weight). One and a half hour after anaesthesia the mean arterial pressure was measured in the common carotid artery by a mercury manometer; a tracheal tube was introduced and the oxygen consumption was measured by a Krogh apparatus applied to dogs. In most tests the cardiac output was determined on the basis of the direct Fick principle and in some by the Stewart-Hamilton dye dilution method. Blood samples were taken from the femoral and pulmonary arteries to determine the arterio-venous oxygen difference. Oxygen saturation and the arterio-venous oxygen difference were determined by an Atlas Universal oxymeter. Evans blue was used in the dye dilution tests. The concentration of dye in the plasma was read at 590 mμ wave length in a Beckman DU spectrophotometer, using a sample obtained immediately before the injection of dye as the control. The determined plasma flow was corrected to whole blood flow on the basis of the haematocrit. Blood withdrawn was replaced by blood obtained from another dog.

After determining the basal cardiac output twice or three times, which served as control values, the animals inhaled an oxygen-poor gas mixture (8—12 per cent oxygen and 88—92 per cent nitrogen) from Douglas bags for 45 to 60 minutes. During this period repeated measurements were made, 50 mg cocarboxylase was injected intravenously and again the cardiac output was determined every tenth minute.

Of the data obtained, those for oxygen consumption, cardiac output and total peripheral vascular resistance (TPR) will be discussed. The levels of cocarboxylase, lactic acid and pyruvic acid will be reported in another paper.

Results

In an attempt to clarify the effects of cocarboxylase on cardiac output under physiologic conditions, i. e. with normal oxygen saturation, the animals were given 50 mg cocarboxylase in four experiments following the determination of control values. It has been found that the cocarboxylase has failed to lead to any changes in cardiac output. In Fig. 1 the cardiac output observed in four experiments is given; the empty columns correspond to the control values, while the squared ones represent the mean values of cardiac output following the administration of TPP. Comparing the respective twin columns, some minor differences can be found, which do not exceed the physiologic fluctuation of cardiac output nor do they go beyond the limits of standard error of the method.

The hypoxic series was divided into two parts. In 18 of the 26 experiments there was a considerable increase in cardiac output as a result of hypoxia, while in 8 cases there occurred some minor changes.

In the experiments where a significant rise in cardiac output, caused by acute hypoxia, has been detected, cocarboxylase reduced it to a level near to the control. The cardiac output values of the 18 experiments are demonstrated by Fig. 2. Each point represents the mean value of an experiment in the control period, in hypoxia, as well as in hypoxia following thiamine-

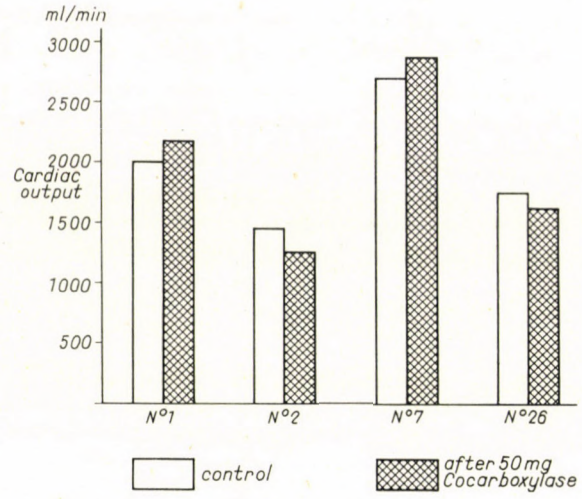

Fig. 1. Effect of 50 mg cocarboxylase on cardiac output under normal arterial oxygen saturation, in four experiments
Empty columns represent the control values of cardiac output; squared columns correspond to the values after cocarboxylase administration

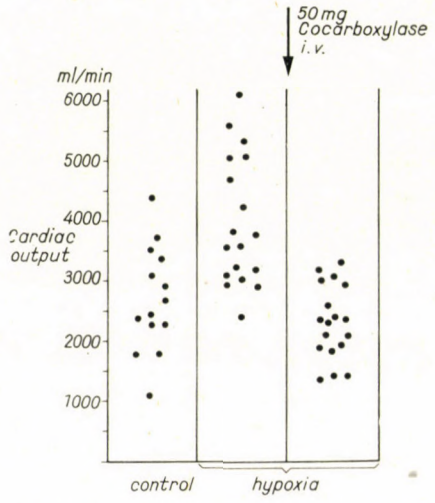

Fig. 2. Effect of cocarboxylase on cardiac output in the first group of hypoxic experiments

pyrophosphate administration. (In four experiments the animals under investigation had already been found spontaneously hypoxic, which might be ascribed to the anaesthesia itself in the control periods, and that is why the normoxic control values are missing.) In Fig. 3 the mean values for cardiac output and oxygen consumption and their standard deviations are given. Compared with the control values, there was an approximately 50 per cent rise in cardiac

output in hypoxia, whereas the increased cardiac output was reduced by cocarboxylase by about 40 per cent, although no essential change occurred in the degree of arterial hypoxia. There was only a slight difference in cardiac output between the control values and those for hypoxia following injection of TPP. Calculating the significance of the differences on the basis of Student's "t" test, the results were as follows. The reduction of hypoxic cardiac output by cocarboxylase was highly significant, $t = 5.20$; $P < 0.001$. The differences between cardiac output values in hypoxia after TPP administration and the control values were $t = 2.36$; $0.05 > P > 0.02$. The average changes in oxygen consumption are also given in Fig. 3. A slight increase was found

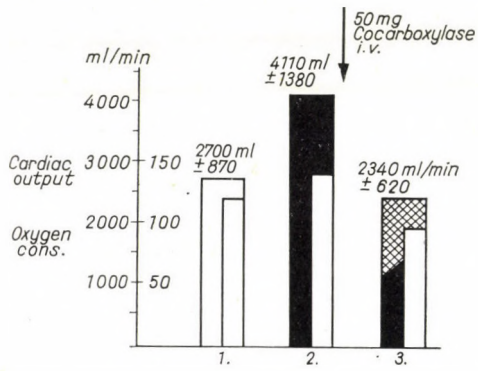

Fig. 3. Changes in cardiac output and oxygen consumption (mean value and standard deviations of 18 experiments) in hypoxia, and in hypoxia after cocarboxylase administration. (Oxygen consumption, empty columns)

in hypoxia; cocarboxylase considerably reduced it. We are inclined to ascribe considerable importance to the latter fact because such a phenomenon could not be detected in experiments where the effects of cocarboxylase have been investigated under normal oxygen saturation. The mean values for oxygen consumption in the respective periods were,

Control	Hypoxia	Hypoxia + TPP
115 ml/min	139 ml/min	89 ml/min

In other words, there was a 21 per cent increase in oxygen consumption during hypoxia, and the cocarboxylase reduced this increased value by 36 per cent. The difference in oxygen consumption between the control and the hypoxic values following cocarboxylase administration was —22 per cent. The decline in oxygen consumption caused by thiamine-pyrophosphate in acute hypoxia was highly significant, $t = 5.74$; $P < 0.001$, and the difference in oxygen consumption between the control and the value in hypoxia following the administration of cocarboxylase was also significant, $t = 2.58$; $0.02 < P < 0.05$.

Changes in peripheral vascular resistance were,

Control	Hypoxia	Hypoxia + TPP
6.560	3.620	5.770 dyne. sec. cm^{-5}

In accordance with data in literature, TPR was found to decrease in hypoxia. Despite hypoxia, under the effect of cocarboxylase administration, TPR increased almost to the level of the control value.

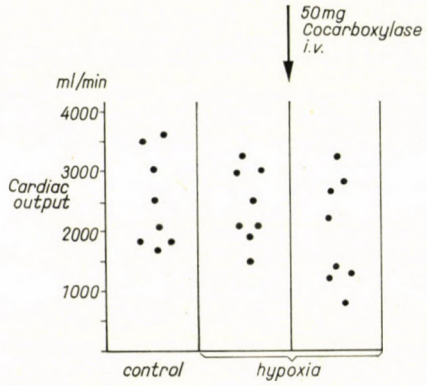

Fig. 4. Effect of cocarboxylase on cardiac output in the second group of hypoxic experiments. (No increase in cardiac output in hypoxia)

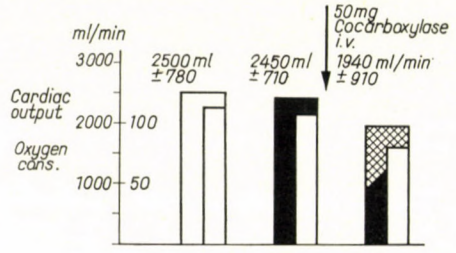

Fig. 5. Changes in cardiac output and oxygen consumption (mean value and standard deviations of 8 experiments) in hypoxia, and in hypoxia after cocarboxylase administration

In 8 experiments there were no remarkable changes in cardiac output during hypoxia. The data of this group of tests are shown in Fig. 4, and the mean values for cardiac output and oxygen consumption are presented in Fig. 5. As seen, there were negligible changes in cardiac output and oxygen consumption in hypoxia, but after thiamine-pyrophosphate administration there occurred a 20 per cent decline in cardiac output, while oxygen consumption decreased by 28 per cent.

In order to determine whether the changes in oxygen consumption and cardiac output taking place after the administration of cocarboxylase were possible consequences of some pseudo-effect (changes which might have happened spontaneously provided hypoxia had been permanent), in 5 experiments the animals inhaled a hypoxic gas mixture without cocarboxylase being injected. The control cardiac output of 2.88 l/min then rose to 4.20 l/min in hypoxia (see Fig. 6), in other words to the same level as in the previous 18 experiments. Oxygen consumption was 130 ml/min under normal conditions, as compared to 135 ml/min in hypoxia. If only the cardiac output values recorded at the end of the hypoxic periods (values corresponding

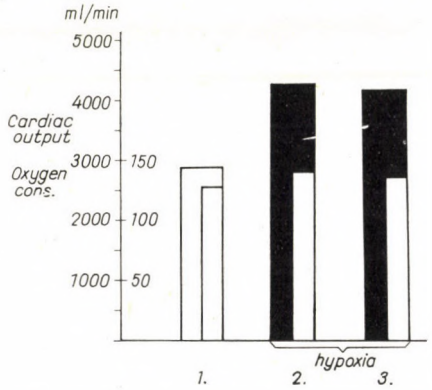

Fig. 6. Effect of permanent hypoxia on cardiac output (the 3rd column corresponds to the respective hypoxia + cocarboxylase phase of the 18 experiments)

to the respective hypoxia + cocarboxylase phase of the above-mentioned 18 experiments) are taken into consideration, the increase of cardiac output was the same as during the first phase of hypoxia where no decrease in cardiac output was detected.

On the basis of the results, the changes taking place after cocarboxylase administration should be attributed to this treatment and can by no means be regarded as subsequent to the depressive circulatory effects of hypoxia.

Discussion

In the majority of our experiments acute hypoxia has been found to induce an increase of cardiac output in keeping with data in the literature. It remains questionable, however, why there was no increase in some of the tests. It seems very likely that differences of individual reactivity were responsible, as it often happens with certain animals and species exposed

to the same treatment. For instance, hypoxia causes no increase in cardiac output in rabbits [16], and a decrease instead of an increase in rats [17]. It is also characteristic that the calculated vascular resistance declines in hypoxia and the arterio-venous oxygen difference narrows. As for metabolism, the rate of oxygen consumption as well as the blood lactic acid level increase in hypoxia. Certain similarities can be found in the changes of circulation and metabolism under the influence of epinephrine and hypoxaemia, both are bringing about an increase of cardiac output and the blood lactic acid level. Lactic acid infusion, which is capable of increasing the blood lactic acid level to the same extent as epinephrine does, causes the cardiac output

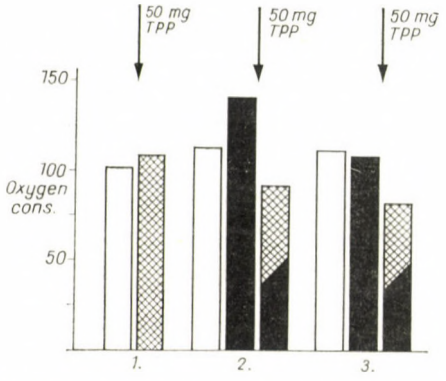

Fig. 7. Effect of cocarboxylase on oxygen consumption under normal oxygen saturation. First column, in the 18 experiments, where there was an increase in cardiac output in hypoxia; second column, in the 8 experiments, where no increase of cardiac output could be detected

also to increase [18]. Presumably view of the effects of epinephrine on circulation and metabolism, arterenol has been suggested to play a role in the circulatory changes occurring under hypoxic conditions [19, 20]. Other investigators, however, have succeeded in producing an increase of cardiac output in dogs in hypoxia following adrenalectomy [5]. By changing the metabolism and through the liberated (and possibly accumulated) metabolites, which induce direct arteriolar vasodilatation, hypoxia may well be susceptible of leading to an increase of cardiac output with or without epinephrine.

The experiments presented have pointed to the role in cardiac output regulation of cocarboxylase, an important factor in intermediary metabolism. We are inclined to presume a relative or absolute lack of cocarboxylase in hypoxia. The present series of experiments points to the possibility that the changes in oxygen consumption may account for the alterations in cardiac output. In an attempt to prove this, we have compiled the data for oxygen

consumption under our various experimental conditions (see Fig. 7). Under normal oxygen saturation, cocarboxylase administration was followed by no significant change in oxygen consumption or cardiac output. Different results have been obtained in the second group of experiments. Here oxygen consumption rose in hypoxia and cocarboxylase administration was followed by a decrease of the raised consumption despite the unaltered hypoxic condition. Similar changes have been observed in cardiac output (see Fig. 3). In the third group there was a slight change in oxygen consumption in hypoxia and a negligible alteration in cardiac output, whereas both were reduced by TPP (see Fig. 5). Our experiments indicate that these changes can be ascribed to the effect of thiaminepyrophosphate, but only under hypoxia. These recall earlier date according to which in heart patients the oxygen debt after exercise can be reduced by cocarboxylase [21].

The authors gratefully acknowledge the technical assistance of K. ALBERT, A. KARAI and V. VAJDA.

LITERATURE

1. HARRISON, T. R., BLALOCK, A., PILCHER, C., WILSON, C. P.: Amer. J. Physiol. **83,** 284 (1927).
2. GROLLMAN, A.: Amer. J. Physiol. **93,** 19 (1930).
3. NAHAS, G. G., VISSCHER, M. B., MATHER, G. W., HADDY, F. J., WARNER, H. R.: J. appl. Physiol. **6,** 467 (1954).
4. KORNER, P. I.: Physiol. Rev. **39,** 687 (1959).
5. GÖMÖRI, P., KOVÁCH, A. G. B., TAKÁCS, L., FÖLDI, M., SZABÓ, GY., NAGY, Z., WILTNER, W., KÁLLAY, K.: Acta med. Acad. Sci. hung. **16,** 93 (1960).
6. GÖMÖRI, P., TAKÁCS, L.: Amer. Heart J. **59,** 161 (1960).
7. LOVEGROVE, T. D., GOWDEY, C. W., STEVENSON, J. A.: Circul. Res. **5,** 659 (1957).
8. JUSTUS, D. W., CORNETT, R. W., HATCHER, J. D.: Circul. Res. **5,** 207 (1957).
9. HIROSHI MIURA: Tohoku J. exp. Med. **19,** 586 (1936).
10. LAHEY, W. J., ARST, D. B., SILVER, M., KLEEMAN, C. K., KUNKEL, P.: Amer. J. Med. **14,** 248 (1953).
11. JONES, JR. R. H.: Circulation **19,** 275 (1959).
12. CAMPBELL, J. A., SELVERSTONE, L. A., DONOVAN, D. L.: J. clin. Invest. **30,** 632 (1951).
13. BRIND, S. H., BIANCHINE, J. R., LEVY, M.: Amer. J. Physiol. **185,** 483 (1956).
14. HAMILTON, W. F.: Circulation **8,** 527 (1953).
15. OCHOA, S.: Biochem. J. **33,** 1262 (1939).
16. KORNER, P. I., EDWARDS, A. W. T.: Quart. J. exp. Physiol. **45,** 113 (1960).
17. TAKÁCS, L.: Personal communication.
18. LUNDHOLM, L.: Acta physiol. scand. **43,** 27 (1958).
19. GREENE, N. M., PHILLIPS, A. D. E.: Amer. J. Physiol. **189,** 475 (1957).
20. BAUGH, C. W., CORNETT, R. W., HATCHER, J. D.: Circul. Res. **7,** 513 (1959).
21. POPPI, A., BARBIERI, L. L., MARTINELLI, M., MARINI, M.: Cardiologia (Basel) **26,** 173 (1955).

Zoltán NAGY
Josephine SKOLNIK
Budapest, VIII. Szentkirályi u. 46, Hungary

THE HUMORAL REGULATION OF PLATELET PRODUCTION*

By

K. RÁK

FIRST DEPARTMENT OF MEDICINE (DIRECTOR: M. JULESZ), UNIVERSITY MEDICAL SCHOOL, SZEGED

(Received May 31, 1962)

A review of the data on the thrombopoietic serum factor is presented, the methods of its demonstration are described and a case material comprising 139 patients is discussed. In mice tests, serum thrombocytopoietic activity was demonstrated with great frequency in polycythaemia, hypersplenism, and acute thrombocytopenia. In patients with thrombocytopenia, normal blood and plasma may also be effective. Questions concerning the factor's source of origin, mode of action, and pathogenetic role, have been discussed. Comparison of the factor with erythropoietin revealed the dissimilarity of the two agents.

For the last ten years the study of the factors governing haematopoiesis has been in the foreground of haematologic research. In general, much attention is being devoted to the questions concerning biological regulations. The haematopoietic system, which hourly supplies vast numbers of cells to the circulation and is at all times capable of adapting supply to widely varying demand, should be possessed of a well-functioning and highly sensitive regulation in order to ensure homeostasis. The study of red cell formation has produced the proof of its humoral control by discovering erythropoietin and establishing its definite role in the regulation. What strikes one is that from the point of view of regulation the other two kinds of peripheral cell should not have been submitted to the investigation they would appear to merit. It is difficult to imagine that the white cells and platelets should lack humoral factors to govern their production. It certainly is no longer permissible to regard erythropoietin and haemopoietin as synonyms of each other, as had been customary to do not so long ago. The question now is how far a theory of the independent regulation of the different cell systems can be veryfied by the data on hand, in other words, is the thrombocytopoietic system also regulated by a factor analogous to erythropoietin, and if so, what essential role has it to fill, and how?

Our knowledge of the fundamentals of the thrombocyte system is but recent; this and the fact that reliable direct methods of platelet counting, eminently phase-contrast microscopy, have become available only a few years ago, explains the comparative paucity of data published. Fonio's indirect

* Presented at the Haematological Congress held in Budapest December, 1961

method, still in current use, lacks accuracy and is of little expediency in serial testing. Widespread though the use of radio-active isotopes is in the study of the kinetics of erythropoiesis, in thrombopoiesis research it is still a matter of the future.

Comprising but a few observations, the literature on the thrombopoietic serum factor is easy to survey (Table 1).

Table 1

Essential literature on the thrombopoietic serum factor

1952	*Stefanini* et. al.	Infusion of polycythaemic blood is followed by remission of thrombocytopenic purpura
1957	*Yamamoto*	Serum from patient after blood loss raises platelet count in rabbits
1958	*Steinberg* et al.	Human albumin fractions cause megakaryocytosis in rabbit
1959	*Linman* et al.	Polycythaemic ether-soluble human serum extract gives rise to pancytosis in the rat; so does batyl alcohol
1960	*Schulman* et al.	Normal human plasma contains thrombocytosis-inducing factor
1958	*Kelemen* et al.	Specific human thrombopoietic serum factor, and its principal properties

At the beginning of the '50s STEFANINI et al. [1] found that blood from polycythaemic donors produced remission in idiopathic thrombocytopenic purpura (I. T. P) Similar observations have since been made by other authors. In addition to the large number of thrombocytes infused, some plasmatic factor has been assumed to have a share in the favourable effect. YAMAMOTO [2] observed the serum of patients after blood loss to raise the platelet count in rabbits. STEINBERG et al. [3] found that certain albumin fractions of the human blood produced megakaryocytosis without simultaneously raising the number of the peripheral thrombocytes. LINMAN et al. [4] induced thrombocytosis in rats using ether-soluble serum extract of human blood. These authors showed that batyl alcohol, isolated by HOLMES from bovine yellow bone marrow, had a similar effect. SCHULMAN et al. [5] demonstrated the presence of a thrombocytosis-inducing factor in normal human serum. A child suffering from congenital deficiency of this factor responded to normal human blood or plasma with thrombocytosis lasting for days and weeks. These same authors reported in the same year some data on a substance of inhibitory effect which was less heatresistant than the stimulating factor. KELEMEN et al. [6] demonstrated a specific thrombopoietic activity from the sera of patients with pathological platelet production, and studied it for its principal properties.

On the basis of our data concerning a polycythaemic patient with thrombocytosis and a thrombocytopenic patient with erythromyelosis, the *principal properties* may be summarized as shown in Table 2.

Table 2

Effective on parenteral application Heat-sensitive pH-sensitive Non-dialysable Runs with the β-globulins Destroyed by trypsin	protein nature, or intimately bound to protein

Administered parenterally, the serum containing the factor increased the circulating platelets in mice; injections of 0.2 to 0.02 ml by the intravenous, and of 0.5 to 0.2 ml by the intraperitoneal route generally produced this effect. Introduced into the stomach, the serum was ineffective. The factor proved heat-sensitive; it largely retained its activity at 4°C for some days, and at room temperature for several hours, but heat of 56°C inactivated it within 60 minutes. A substantial shift of the pH towards either alkalinity or acidity likewise rendered the factor inactive. The dialysing membrane retained it, in electrophoresis it ran with the beta-globulins, and trypsin destroyed it. All these properties seem to point to its being of protein nature or intimately bound to protein. As already mentioned, it is effective in small amounts, i. e. at high dilutions, and is inactivated on incubation with normal human serum at the ratio of 1: 1. In view of these latter qualities the factor's effect might be assumed to be enzymatic in nature.

For its incidence the factor was studied in patients treated during the last three years in this Department and in the Postgraduate Medical School in Budapest. The examinations in the latter institute were carried out by E. KELEMEN and D. LEHOCZKY. In this Department I. CSERHÁTI and F. KRIZSA helped to collect the data.

Groups of 4 to 6 mice were injected with 0.2 ml of fresh serum intravenously, or with 0.5 ml intraperitoneally. Using FEISSLY and LÜDIN's direct method of phase-contrast microscopy as modified by FISCHER and GERMER [7], platelet counts were made immediately before and three, respectively five, days after the injection. When the average rise in the platelet count exceeded 30 per cent the result was denoted as positive, if not, then as negative. When the rise was substantial in but a proportion of the mice in the group, the result was regarded as questionable ($\pm$). Whichever of the 3-day and 5-day counts showed the higher increase in the number of platelets, was taken to be the standard; in most cases it was the 5-day count.

The results obtained with sera from 139 patients are presented in Table 3.

Table 3

Thrombocytopoietic activity of sera from patients with pathological and normal platelet production in mice

Clinical diagnosis	Number of patients	Result		
		+	±	−
Thrombocytosis in				
polycythaemia vera	10	8	2	—
Other types of thrombocytosis	13	4	2	7
Thrombocytopenia in				
acute I. T. P.	4	4	—	—
chronic I. T. P.	11	1	3	7
secondary hypersplenism	16	10	4	2
haemoblastosis, myelofibrosis, pancytopenia, carcinomatosis	27	4	5	18
Normal platelet count in				
polycythaemia vera	6	—	—	6
chronic erythromyelosis	2	2	—	—
other blood diseases	17	5	2	10
Healthy or haematologically normal subjects	33	—	2	31
	139	38	20	81

+ = positive, ± = questionable
— = negative

Mostly patients with pathological platelet production were examined. The group of subjects with normal thrombocytopoiesis served as control. Grading was done on the basis of the peripheral platelet counts. Among the diseases associated with *thrombocytosis* the first place was held by polycythaemia vera; all cases were positive or questionable. In thrombocytosis following splenectomy, or some unknown cause, the results differed in grading. Of the diseases involving *thrombocytopenia*, all cases of acute I. T. P. were positive, but most of those with chronic I. T. P. were negative. The group of secondary hypersplenism included cases of cirrhosis, congestive splenomegaly, Felty's syndrome, and Gaucher's disease and was conspicuous for the great proportion of positive and questionable cases. In the greater part of the blood diseases, accompanied by a reduction in the number of platelets, the serum showed no activity nor the sera of patients with advanced polycythaemia with *normal platelet count*. The sera of two patients with chronic erythromyelosis could be, on the other hand, shown to contain the factor. In the group of subjects with physiological haemopoiesis all but two questionable cases were negative. Although no general principle can be inferred from the evidence in Table 3, it is not without interest that most of polycythaemia associated with thrombocytosis, of acute I. T. P., and of secondary hypersplenism, were positive, whereas the greatest part of the patients with

thrombocytosis, chronic I. T. P. and other conditions involving a decrease in the platelets yielded negative results.

With the method outlined it was not possible to demonstrate the presence of a thrombocytopoietic factor in the serum of healthy individuals. The absence of a positive reaction might have been due to the moderate sensitivity of the method. We once more refer to SCHULMAN et al. [5] who found that plasma from normal donors elicited thrombocytosis in a child with blood platelet deficiency. Repeated attempts have been made to influence the platelet count in patients with I. T. P. by blood or plasma infusions. That normal blood preparations can influence the number of platelets in the circulation, is demonstrated in Fig. 1.

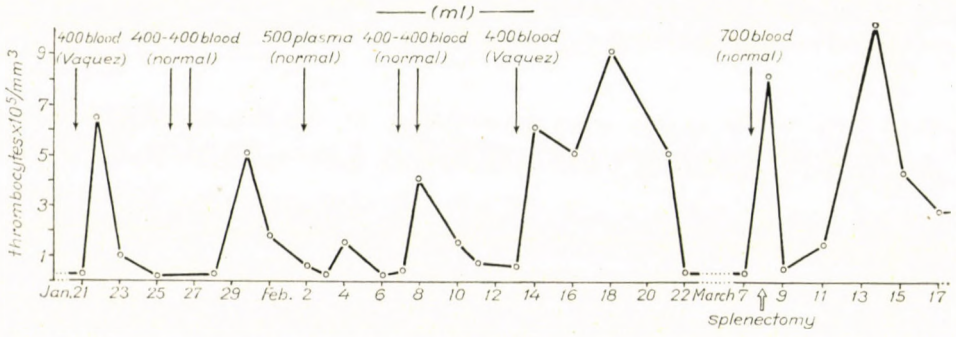

Fig. 1. Platelet count of patient with I. T. P. following administration of polycythaemic blood, normal whole blood, and normal plasma

A patient with chronic I. T. P. had a platelet count of about 30.000, Repeated infusions of polycythaemic blood, normal blood and plasma. were followed by considerable increases in the number of platelets; the increases were higher after blood from polycythaemic donors. The change was not due to the thrombocyte infused, for in many occasions the platelet count remained unchanged for several hours after the infusion. The thrombocytosis elicited lasted 2 to 8 days. Splenectomy had a favourable effect.

In another patient the changes were less marked. In one instance, polycythaemic blood, confirmed positive by the mouse test, proved to be ineffective in amegakaryocytic thrombocytopenia (malignant reticulosis). The data seem to favour the view that normal plasma, too, possesses thrombocytopoietic activity, though our moderately sensitive method is unable to disclose it.

Thrombocytopoietic activity was often found to vary in serum samples from the same patient. It was found to weaken and gradually cease with the progression of the disease. This was particularly the case in polycythaemia. The sera of polycythaemic patients with normal platelet count were usually

negative. Several cases with hypersplenism ceased to yield positive results after splenectomy. Nor were drugs without effect; for instance, prednisone was found capable of completely suppressing activity. It has to be noted that so far we have made no efforts at quantitative determinations.

As regards the *origin* of the factor, at best only evidence of a negative nature is available in relation to humans. *E. g.*, the possible presence of activity after splenectomy denies the spleen's playing an essential role. The factor's analogy to erythropoietin merely points to the necessity of further animal experiments to approach the problem. In mice, thrombocytosis following treatment with ultraviolet rays is brought about by a humoral factor, and this has been found to resemble the one in man in many of its properties [8]. X-ray irradiation has been shown to depress or suspend the formation of this factor, which we may term the UV-thrombocytopoietic factor [9]. It seems not unreasonable to conclude that some radio-sensitive cell system or organ comes into play when it is formed [9]. In mice, the factor has been found to be independent of the presence of the spleen for its formation.

In studying the *mechanism of action*, difficulties of a technical nature are inescapable. To examine, with confidence in the results, the quantitative conditions of the megakaryocyte system of experimental animals, which with us are mice, is an intricate task. Cytological and histological examination of bone-marrow smears, and to an even greater extend of bone-marrow sections, have so far failed to produce readily evaluable and numerically demonstrable results; in other words, a hyperplasia of megakaryocyte system still awaits confirmation. In the above-mentioned thrombocytosis elicited in mice with UV-irradiation, both the bone marrow and the number of megakaryocytes in the spleen were taken into account, and splenic megakaryocytosis was demonstrated. Should one at all expect the thrombopoietic activity to increase the number of megakaryocytes in the bone marrow? There are data in the literature to underline this question. MATTER et al. [10] showed in rats that rises in the platelet count following thrombocytopenia induced by exchange transfusion, are accompanied only by moderate, not more than about 20 per cent, increases in the number of megakaryocytes; they also showed that the moderate changes were those revealed by electron microscopy in the cytoplasm. In addition to those referring to the bone-marrow effect, there are data in the literature which support the view that the thrombocytes appearing in the circulation must be newly-formed cells since, unlike in the erythrocyte and leukocyte systems, in the thrombocyte system there are no, or at least no substantial, reserves, there exists no platelet pool. The observations made by KREVENS et al. [11] in humans and by CRADDOCK et al. [12] in animal experiments, favour this view. A similar standpoint is occupied by ROHR [13]. In the present experiments, the rise in the number of thrombocytes took several hours, more often days,

to develop and commonly lasted 6 to 8 days, ceasing gradually. It was certainly no rapid, transitory phenomenon. And so the study of the mechanism of action is still far from complete; further systematic investigations are needed, including examinations of bone-marrow cultures. Concerning the possible part of the spleen in the mechanism, only negative observations could be made; activity was found not to depend on the presence of this organ. On the contrary, positive human serum increased the platelet count at a slightly higher rate in the splenectomized than in normal mice.

What is the *pathogenetic role* of the factor? Clinical observations do not suffice to answer the question, clarification of both the source of origin and the pathogenetic role must be expected from animal experiments. The results of some earlier experiments of ours suggest a role of serum activity in the changes of circulating platelet count [14, 15]. Mainly these results would seem to justify the assumption that whenever the thrombocyte system becomes deranged, one (or more) humoral factor(s) comes into play to restore the thrombopoietic equilibrium. In this connection, the observation of DUX et al. [16] merits attention; they found that the serum of infants who had become thrombocytopenic in consequence of exchange transfusions before normalization of the own's thrombocyte count, was capable of increasing the number of platelets in mice. This finding, too, points to causative relations between serum activity and changes, more precisely increases, in the number of circulating thrombocytes.

The question has to be raised how the thrombopoieses exchanging factor is related to *erythropoietin*. In our view, the two factors are not identical, and numerous data and clinical observations have shown differences between them. Similarities nevertheless also occur (Table 4). Investigations to thrombopoietic sera have revealed data for which, to our knowledge, the erythropoietic serum factor has not yet been studied. These data offer no support for LINMAN's standpoint that all three cell systems are governed by a common haemopoietin. For instance, erythrocytosis of renal origin associated with kidney tumours is not accompanied by thrombocytosis in spite of the high erythropoietin titre.

Finally, may the factor under discussion be regarded as a poietin? May one speak of *thrombopoietin* in connection with it?

In our opinion, what we are dealing with is indeed a substance with specific effect on thrombopoiesis. Nevertheless, there being no indisputable proof of the specific response of the megakaryocyte system, we have generally avoided use of the term thrombopoietin. Our ignorance of the chemical nature of the changes occurring in the primitive bone-marrow cell might be excused on the ground that though detected more than half a century earlier, erythropoietin is not very well known either. More knowledge of it would certainly be conducive to the clarification of its enzymic nature.

Table 4

Principal properties of the human erythropoietic and thrombopoietic serum factors

Property	E. S. F.	T. S. F.
Demonstrability		
in cytosis	yes (?)	yes
in cytopenia	yes	yes
Activity weakens and gradually ceases during normalization of cell count	yes	yes
Heat sensitivity	absent	present
Sensitivity to shifts in pH	moderate	marked
Destroyed by trypsin	yes	yes
Retained by dialysing membrane	yes	yes
In electrophoresis runs with	α-globulins	β-globulins
Chemistry	glycoprotein	—
At high dilutions	—	effective
Inhibited when mixed with normal serum	—	yes

Like all problems of biological regulation, those of the humoral regulation of haemopoiesis are intricate problems. Apart from its theoretical importance, their study promises to disclose future possibilities of diagnostic and, even more so, of therapeutic value.

The evidence on hand appears to show:

(i) that there most probably exists a thrombopoietic factor;

(ii) that this factor probably contributes to restore physiological platelet production whenever there is a sudden and considerable interference with the thrombocyte system. The available data are inadequate to decide whether the factor participates in normally balanced thrombopoiesis;

(iii) most kinds of thrombocytopenia are due to some cause other than the absence of the factor. The reason why poietinaemia associated with these conditions is devoid of effect, respectively why at other times no poietic activity is present despite severe cytopenia, is unknown;

(iv) although in certain types of thrombocytosis the serum is demonstrably active in inducing cytosis, its participation in maintaining lasting thrombocytosis cannot be established on the basis of the data at disposal.

REFERENCES

1. Stefanini, M., Chatterjea, J. B., Dameshek, W., Zannos, L., Santiago, E. P.: Blood **7,** 53 (1952).
2. Yamamoto, S.: Acta haemat. jap. **20,** 163 (1857).
3. Steinberg, B., Dietz, A. A., Atamer, M. A.: Arch. Path. **67,** 496 (1958).
4. Linman, J. W., Bethell, J. F., Long, M. J.: Ann. intern. Med. **51,** 1003 (1959).
5. Schulman, I., Pierce, M., Lukens, A., Currimbhoy, J.: Blood **16,** 943 (1960).

6. KELEMEN, E., CSERHÁTI, I., TANOS, B.: Acta haemat. **20,** 350 (1958).
7. FISCHER, W., GERMER, W. D.: Röntgen-Lab. Prax. **10,** 49 (1957).
8. CSERHÁTI, I., RÁK, K.: Z. ges. exp. Med. **133,** 38 (1960).
9. CSERHÁTI, I., KRIZSA, F., SÖVÉNYI, E., RÁK, K.: Z. ges. exp. Med. **135,** 355 (1962).
10. MATTER, M., HARTMANN, J. R., KAUTZ, J., DEMARSH, Q. B., FINCH, C. A.: Blood **15,** 174 (1960).
11. KREVANS, J. R., JACKSON, D. P.: J. amer. med. Ass. **159,** 171 (1955).
12. CRADDOCK, C. G., ADAMS, W. S., PERRY, S., LAWRENCE, J. S.: J. Lab. clin. Med. **45,** 906 (1955).
13. ROHR, K.: Das menschliche Knochenmark. Thieme, Stuttgart 1960.
14. RÁK, K., KRIZSA, F., CSERHÁTI, I.: Med. exp. (Basel) **5,** 91 (1961).
15. KRIZSA, G., CSERHÁTI, I., RÁK, K.: Med. exp. (Basel) **7,** 32 (1962).
16. DUX, E., KOVÁCS, Z., GIMESY, G.: Acta haemat. **27,** 334 (1962).
17. LINMAN, J. W.: J. Lab. clin. Med. **59,** 262 (1962).

Kálmán RÁK, Szeged, First Department of Medicine, University Medical School, Hungary

EEG CHANGES INDUCED BY ELECTROSHOCK LOADS IN SCHIZOPHRENICS

By

I. Huszák and I. Somogyi

DEPARTMENT OF NEUROPSYCHIATRY AND INSTITUTE OF BRAIN RESEARCH, UNIVERSITY MEDICAL SCHOOL, SZEGED

(Received July 10, 1962)

On the basis of electroencephalograms recorded from schizophrenics and non-schizophrenics during the first treatment with electroshock the following may be established.

1. In the controls the normalization of the electroencephalograms took place within 10 to 15 minutes after the load and occurred in a steep straight line.

2. In schizophrenics this process lasted 35 to 40 minutes or even longer.

3. The conclusion may be drawn that, owing to the retarded normalization period and the intermittent relapses, in schizophrenics the functional capacity of the metabolic processes of the brain maintaining the functioning structures is disturbed.

Recently numerous authors have dealt with the question of the possibility of analysing electrophysiologically normal and pathologic psychic functions. The results are not unequivocal. So far the attempt to find relations between the intellectual level, the neurotic, psychopathic and psychotic conditions, respectively, and the associated electroencephalographic changes has not been successful.

The opinion of most authors is that the dysrhythmic irregular electrical activity is striking in the electroencephalographic curves of the different psychiatric diseases (Review of Henry and Knott [10]).

The evaluation of the schizophrenic electroencephalograms by the different authors vary to a great extent. According to Gibbs [8] the electroencephalogram of schizophrenics does not considerably deviate from the normal variant. Gastaut [6], Schwab [16], Bickford [1], Kershman [11], Lieberman et al. [13] have observed that the convulsive threshold against pentamethylene tetrazol is decreased in schizophrenics. Lefman and Perlo [12] using combined photo-metrazol tests a pathologic EEG activity in 53 per cent of schizophrenics. Failla [5], Goldmann [9] found pathologic EEG phenomena similarly in schizophrenic psychotics under anaesthesia. Magyar [14] observed similarly severe pathologic changes after a small dose of pentetrazol.

Callaway and Boucher [2] were the first to give an account of EEG changes evoked by shock treatment. They observed the delta activity which appears following convulsive treatment often to persist for a period of 3—4

months. They could not demonstrate a definite correlation between the degree of recovery and the persistence of delta waves.

TSUGUNOV and NICOLAEV [4] believe that the appearance of the delta waves is indicative of brain damage and therefore warn with regard to electroshock treatment. MARTINO [15] detected the delta wave activity caused by electroshock mainly in the fronto-precentral and fronto-basal regions. Neither could CHUSHID and PACELLA [3] find a relation between the clinical improvement and the disappearance of the delta waves. They observed, however, that after few electroshock treatments the delta activity disappeared earlier than if the shock treatments were more numerous and examined therefore the correlation between the number of electroshock treatments and the period of EEG irregularity.

In the course of our studies concerning schizophrenia we have attempted to obtain data not only about the metabolic changes of the schizophrenic organism, but also about the functional conditions of the nervous system of schizophrenics. For this purpose the examination of the EEG changes following functional loads of cerebral bioelectric activity seemed suitable. The present paper gives an analysis of the EEG changes following electroshock loads in schizophrenics.

Methods

The examinations were carried out in 20 schizophrenics and 6 control psychopaths between 20 and 40 years of age. Mainly acute cases not yet subjected to treatment were examined. We had only a few cases where some years previously electroshock therapy had been applied. Drugs were withhold for several days before the examination. During this time as well as before the application of the electroshock the EEG was repeatedly recorded with the patients at rest. Electroshock was carried out with a Siemens Electroconvulsator apparatus (on the average 500 mA, 0.5 sec), by means of bitemporally placed electrodes. Previously bifrontally, bitemporally and biparietally electrode wires were fixed on the head of the patients. The recordings were made with an 8 channel Kayser ink writing electroencephalograph. Recording was started ½ to 1 minute after the electro-convulsions had ceased. Generally after fixation of the first initial phase every 5 minutes a short phase was recorded. The recordings were carried out for 40 to 60 minutes, until the frequency and average amplitude of the curve attained the pattern seen before the shock treatment. In each patient only the first electroshock was taken into account. Evaluation was performed in the following manner. All the waves were counted in intervals of 30 seconds, and from these the number of waves for 1 second was calculated. The delta waves of the same phases were also counted and their number for one second was established. Thus the time of appearance and arrangement of the different frequencies as well as the delta waves following electroshock loads were examined.

Results

The results may be summarized as follows. In the controls after the electroshock load the initial, almost isoelectric, short "silent" phase was replaced by a slow delta phase with a 600 to 1000 μ V amplitude of 0.5—1 c/s alternating with short flat phases. Five minutes later a gradual shift of

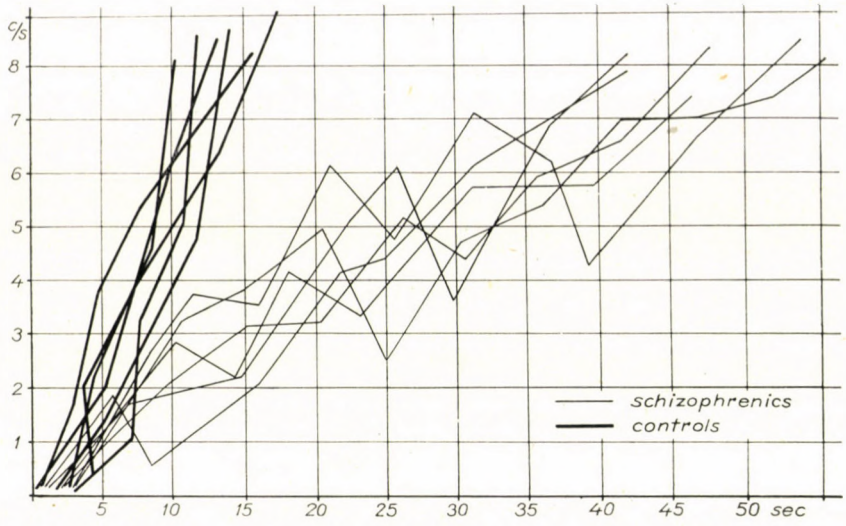

Fig. 1

the frequency could be observed and the appearance of delta waves diminished. Subsequently the delta waves disappeared and usually within 10 to 15 minutes complete rearrangement took place, the frequency returned to the initial value. The increase of frequency showed a steep rise without falling back (Figs. 1 and 2).

In the EEG curves of schizophrenics the bioelectric changes showed a different pattern (Figs. 3, 4, 5, 6, 7). The initial period practically agreed with that of the controls. However, the decrease in the number of delta waves, the shortening and disappearance of the initial silent and the flat phases, as well as the increase of the frequency ensued much later and more slowly.

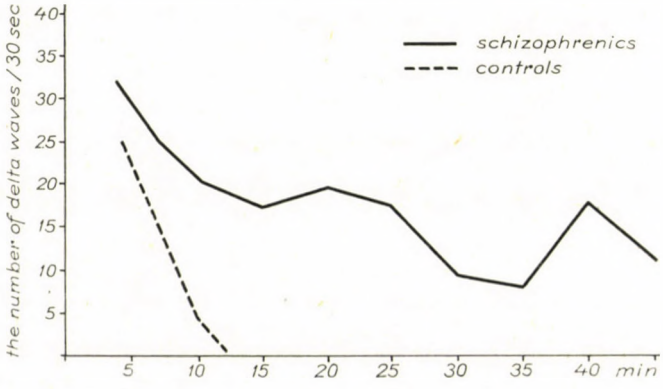

Fig. 2

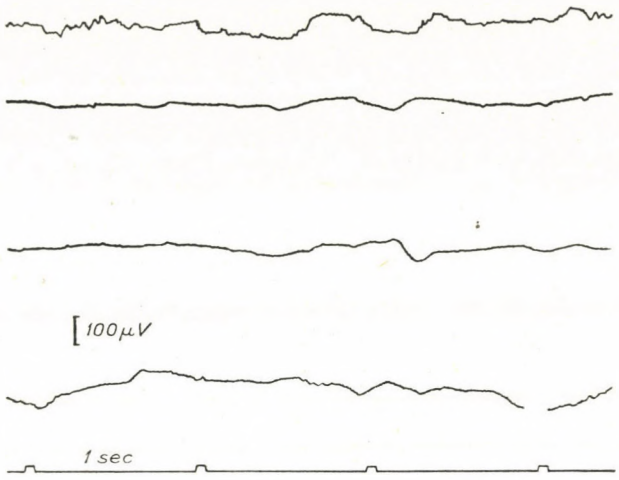

Fig. 3

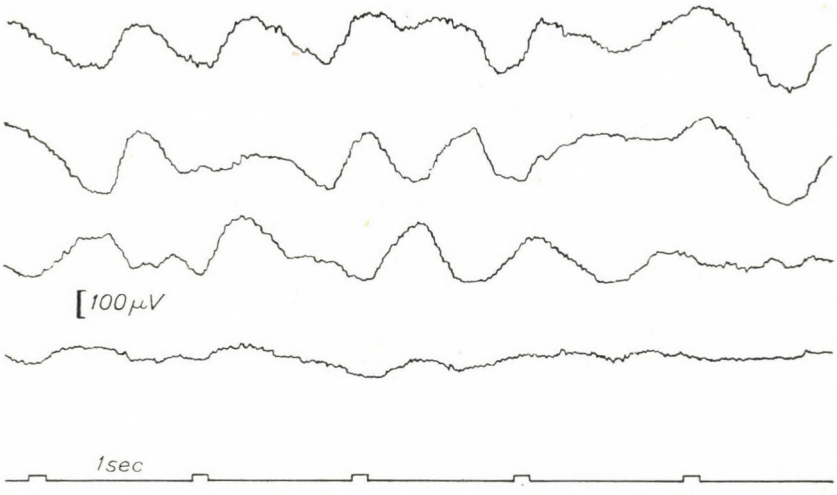

Fig. 4

In all cases relapses mainly concerning the frequency (decrease of frequency) could be observed, but meanwhile the number of the delta waves increased. The restoration of the curves corresponding to the starting pattern occurred only after 35—50 minutes. In some cases rearrangement took place even later (see Figs. 1, 2 and the EEG curves).

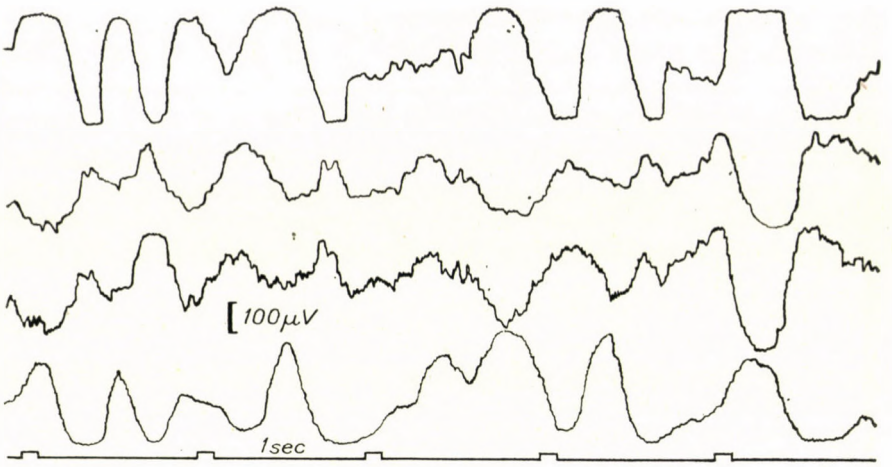

Fig. 5

Discussion

The results obtained show the striking fact that after the first electroshock load schizophrenics display prolonged functional disturbances as compared to the controls. Furthermore, the EEG curves of schizophrenics were characterized by frequently alternating phases of improvement and relapse. These data imply that the schizophrenic brain responds differently — in the present case to electroshock loading — from the non-schizophrenic one. Rearrangement occurs later and is not continuous, but fluctuates showing

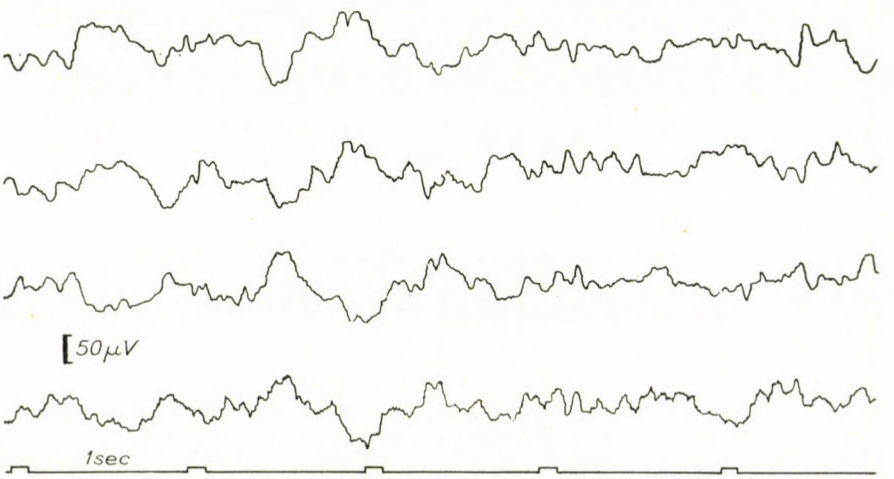

Fig. 6

sometimes a competition of the different functional states. In addition to the persisting delta waves attention must be paid to the more prolonged isoelectric, so-called "silent", phases lasting over a longer period. They would namely represent from the electrophysiological aspect a state of inactivity or inhibition, respectively; this was also experienced in animal experiments. This periodical and fairly prolonged inhibitory phase may in our opinion be considered as the exhaustion and a manifestation of the protective inhibition of the cortex. This was more significant in the schizophrenic brain than in that of the controls.

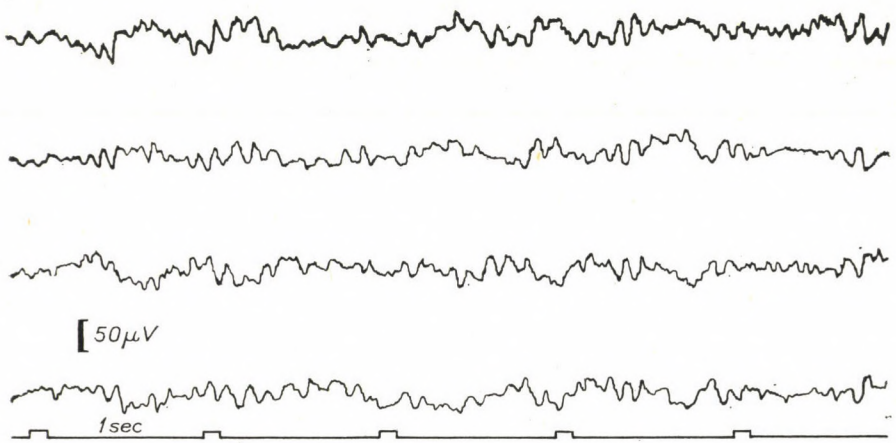

Fig. 7

Changing our trend of thought from the electrophysiological point of view to biochemical conditions it must be assumed that the metabolic processes which restitute or repolarise the functional membranes operate far slower in schizophrenics than in the controls. Namely, it is well known that the functional state of the nerve cell is regulated by the activity of its energy-producing oxidation system. The energy-producing oxidation processes functioning kinetically with a suitable intensity can repair the metabolic disorders occurring after too strong functional demands far quicker than the oxidation systems with lower intensity and defects.

The above suggest that the analysis of cerebral bioelectric activity recorded in the course of loadings renders possible to obtain certain data concerning the functional capacity of the metabolic processes.

REFERENCES

1. Bickford, R. G.—Daly, D.: Experiences with the photo-metrazol test. EEG. Clin. Neurophysiol. **3,** 378 (1951).
2. Callaway, E. and Boucher, F.: Slow wave phenomena in intensive electroshock. EEG. Clin. Neurophysiol. **2,** 157 (1950).
3. Chushid, J. G.—Pacella, B. L.: The electroencephalogram in the electric shock therapies. J. Nerv. Dis. **116,** 95 (1952).
4. *Гугунов Ш. А. — Николаев Ю. Ш.*: Невропот и псих. 19, 59 (1950).
5. Failla, E.: Acta Neur. Napoli, **12,** 319 (1957).
6. Gastaut, H.: Combined photic and Metrasol activation of the brain. EEG. Clin. Neurophysiol. **2,** 249 (1950).
7. Gibbs, F. A.: Abnormal electrical activity in the temporal regions and its relationship to abnormalities of behaviour. XXXVI Proc. Ass. Res. J. Nerv. Ment. Dis. **10,** 278 (1958).
8. Gibbs, F. A.: Ictal and non-ictal psychiatric disorders in temporal lobe epilepsy. J. Nerv. Ment. Dis. **113,** 522 (1958).
9. Goldmann, D.: EEG. Clin. Neurophysiol. **11,** 4 (1959).
10. Henry, Ch. E. and Knott, J. R.: Progr. Neur. Psych. **14,** Chapter 13 (1959).
11. Kershman, J.: Combined photic-Metrasol activation in normal subjects and in psychoneurotic and schizophrenic patients. EEG. Clin. Neurophysiol. **3,** 377 (1951).
12. Lefman, H.—Perlo, V. P.: Metrasol and combined photic-Metrasol activated EEG in epileptic, schizophrenic, psychoneurotic, and psychopathic patients. EEG. Clin. Neurophysiol. **7,** 61 (1955).
13. Lieberman, D. M.—Hoenig, J.—Hacker, M.: The metrasolflicker threshold in neuropsychiatric patients. EEG. Clin. Neurophysiol. **6,** 9 (1954).
14. Magyar, I.: Acut schizophren psychosisok kóros electroencephalographiás jelenségeinek értékelése. Ideggyógy. Szle **14,** 44 (1961).
15. de Martino, U.: L'Elettroencefalografia nell annichilimento. Rass. neuropsychiat. **5,** 112 (1951).
16. Schwab, R.: EEG in clinical practice. Saunders, Philadelphia, 1951.

István Huszák, István Somogyi | Szeged, Dep. of Neuropsychiatry, University Medical School, Hungary

UNTERSUCHUNGEN MIT J^{131} MARKIERTEM TRIOLEIN BEI ATHEROSKLEROTIKERN

Von

G. Erdélyi, L. Keller und I. Balázsi

III. MEDIZINISCHE KLINIK DER MEDIZINISCHEN UNIVERSITÄT BUDAPEST (DIREKTOR: PROF. DR. S. GERŐ

(Eingegangen am 25. Juli 1962)

Die Aktivitätskurve des peroral verabreichten J^{131} Triolein-Monochlorids wurde im Vollblut und im Trichloressigsäurepräzipitat untersucht. Bei atherosklerotischen Kranken ist die Kurve höher und fällt langsamer ab, als bei normalen Personen. Die Aktivitätswerte der Kranken, bei denen der Verdacht einer Koronarsklerose bestand, aber diese Krankheit war mit üblichen klinischen Untersuchungsmethoden noch nicht nachweisbar, lagen teils den Normalwerten, teils den Werten der atherosklerotischen Gruppe näher. Auf Grund der Literaturangaben und der eigenen Ergebnisse wird angenommen, daß die Methode in der Frühdiagnose der Atherosklerose nutzbar sein könnte.

In den letzten Jahrzehnten ist die Atherosklerose eines der Zentralprobleme der Medizin geworden. Die klinische Erforschung ist jedoch durch die mangelhaften, nicht zuverlässigen und oft komplizierten diagnostischen Verfahren erschwert. Da der Zusammenhang zwischen Atherosklerose und Lipoidstoffwechsel seit langem bekannt ist, beruht ein bedeutender Teil der diagnostischen Verfahren auf den Veränderungen der Blutlipoide. Die weitläufig angewandten Se-Cholesterin, Gesamtlipoid, β-Lipoprotein, Phospholipid, usw. Bestimmungen zeigen meistens statistisch gut bewertbare Abweichungen, bieten jedoch zur Diagnosestellung der einzelnen atherosklerotischen Kranken keinen genügenden Anhaltspunkt. Die Anwendung von mit radioaktiven Isotopen markierten Fetten eröffnete eine neue Möglichkeit für die Untersuchung der Dynamik des Fettstoffwechsels. Stanley und Thannhauser [1] konnten als erste die Resorption des mit J^{131} markierten Trioleins und die Elimination der Aktivität aus dem Blut bei Nephrotikern und hyperlipämischen Kranken beobachten und im Vergleich zum Normalwert eine signifikante Veränderung feststellen. Des weiteren fand das Verfahren zum Studium der mit Lipoidstoffwechselstörungen verbundenen Erkrankungen ausgedehnte Anwendung [2—12]. Likoff und Mitarb. [22, 23] fanden nach peroraler Verabfolgung von J^{131}-Triolein im Blut bzw. im mit Trichloressigsäure gewonnenen Eiweißpräzipitat der atherosklerotischen Kranken, im Vergleich zu den normalen Kontrollpersonen eine höhere und langsamer abfallende Aktivität. Ähnliche Resultate erhielten auch Seller und Mitarb. [24]. Veränderte Aktivitätskurven konnten neustens Sandberg und Mitarb. [25] bei Diabetes mellitus, Hakkila und Mitarb. [26] bei kar-

diale Dekompensation, CROFFORD und Mitarb. [27], SIGLER und RUBINI [28], sowie MENG [29] bei essentieller Hyperlipämie registrieren. METZ und Mitarb. [30] fanden bei Bantu-Negern, SCHWARTZ und LEWITUS [31] bei Juden jemenischer Abstammung niedrigere Aktivitätskurven als bei den Weissen bzw. Europäern. Das Verfahren fand auch bei der Bewertung der Wirkung von blutlipoidvermindernden Medikamenten eine Anwendung [32—35].

Die Zielsetzung unserer Untersuchungen war, neue Angaben über die Anwendbarkeit dieser Methode in der Diagnosestellung der Atherosklerose zu gewinnen.

Material und Methodik

Die Untersuchungen erfolgten an 3 Krankengruppen: In die erste Gruppe (10 Fälle: 8 Männer, 2 Frauen, Lebensalter 55—73 Jahre) wurden die Patienten gereiht, bei denen auf Grund der Anamnese, der klinischen, Laboratoriums- und EKG-Befunde sicher Atherosklerose vorlag. Sämtliche dieser Patienten litten an schwerer Koronarsklerose, bei 8 Kranken war in der Anamnese sogar ein Myokardinfarkt vorzufinden. Bei den Patienten der zweiten Gruppe (Kontrollgruppe: 10 Fälle: 6 Männer, 4 Frauen, Alter zwischen 15—37 Jahren) waren keinerlei Symptome der Atherosklerose bzw. einer anderen mit Lipoidstoffwechselstörung verlaufenden Erkrankung zu finden. Zur dritten Gruppe gehörten die Patienten (4 Männer, 2 Frauen, 40—48 Jahre alt) die zwar auf Grund der Anamnese (anginöse Schmerzen) auf Koronarsklerose verdächtig waren, aber diese Krankheit mit den üblichen Untersuchungsmethoden nicht nachgewiesen werden konnte. Kranken mit Resorptionsstörungen (Gallenblase-, Leber-, Magen-, Pankreas-, Darmkrankheiten) oder kardial Dekompensierte enthielt keine der Gruppen, da bei letzteren infolge der Stauung die Resorption unvollkommen ist [26].

Um die Jodaufnahme der Schilddrüse zu hemmen, wurde den Patienten vor der Untersuchung 3 Tage lang täglich 3×5 Tropfen Lugol-Lösung verabreicht. Am Tag der Untersuchung erhielten die Kranken auf nüchternen Magen ein Gemisch von 0,5 ml/kg Körpergewicht Speiseöl und 3 dl Milch, das J^{131}-Triolein-Monochlorid von 50—80 μC Aktivität enthielt. Nach 2, 4, 6, 8 und 24 Stunden wurde von den Patienten je 15 ml Blut in ein Heparin enthaltendes Reagenzglas entnommen, das bis zur Bestimmung im Kühlschrank aufbewahrt wurde. Die Patienten durften zuerst nach der 4stündigen Blutentnahme Nahrung zu sich nehmen.

Zur Blutvorbereitung wurde mit einigen Modifikationen die von BERES und Mitarb. [8] empfohlene Methodik angewendet. Nach kräftigem Aufschütteln wurden aus den Heparin enthaltenden Blutproben mit Hilfe eines manuellen Pipettierapparates für die Bestimmung der Aktivität des Vollblutes je 2 ml in Reagenzgläser gemessen. Zur Isolierung der Lipoproteinfraktion wurden je 3 ml in Zentrifugengläser pipettiert und unter ständigem Rühren zuerst mit 5 ml 10%igem Kaliumjodid, dann tropfenweise mit 4 ml 40%iger Trichloressigsäure versetzt. Man läßt das Gemisch 30 Minuten lang stehen, rührt mit einem Glasstab um und zentrifugiert 10 Minuten mit 5000 Umdrehungen pro Minute. Die Supernatans wird verschüttet, zum Niederschlag gibt man unter ständigem Rühren 6 ml 10% iges Kaliumjodid, sowie tropfenweise 2 ml 40%ige Trichloressigsäurelösung, dann wird abermals 10 Minuten lang zentrifugiert. Das Verfahren wird so lange wiederholt, bis die Aktivität der Supernatans beträgt weniger, als das 1,5fache der Hintergrundaktivität. Die Aktivität des auf diese Weise gewonnenen Präzipitats wird im Bohrlock-Kristall von 2 ml Kapazität gemessen. Die Aktivität des Vollblutes und des Trichloressigsäurepräzipitats wurde auf das Gesamtblutvolumen (7,7% des Körpergewichtes) umgerechnet und in Prozenten der verabreichten Trioleinaktivität ausgedrückt. Die Berechnung der Verabreichten Impulse erfolgte auf Grund der Impulse der am Versuchstag hergestellten 1 : 250 in Benzin verdünnten Lösung des J^{131}-Triolein-Speiseölgemisches.

Ergebnisse

Die Resultate der Normalen und der Atherosklerotiker sind in Abb. 1—6 und Tab. I dargestellt.

Die 2- und 4stündigen Werte der beiden Gruppen sind im wesentlichen übereinstimmend. Der Durchschnitt der 6stündigen Resultate zeigt bereits bedeutende Abweichung: Im Gegensatz zum fast unveränderten Niveau der Kontrollkurve zeigt die Durchschnittskurve der atherosklerotischen Gruppe ausdrückliche Erhöhung. Dieser Unterschied zwischen den zwei Gruppen st bei den 8stündigen Werten noch signifikant. Die 24stündigen Werte nähern

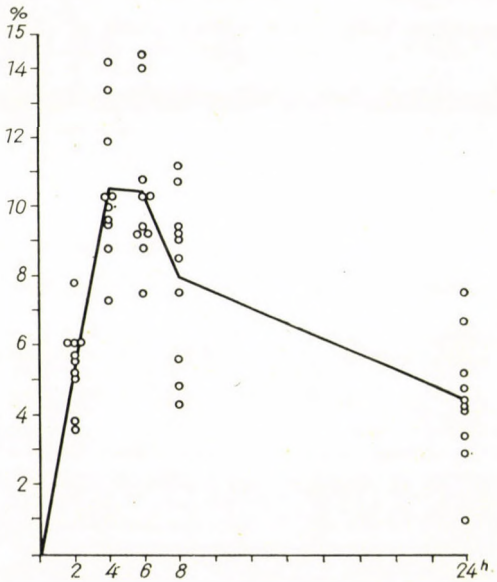

Abb. 1. Vollblutaktivitätswerte und Durchschnittskurve normaler Personen

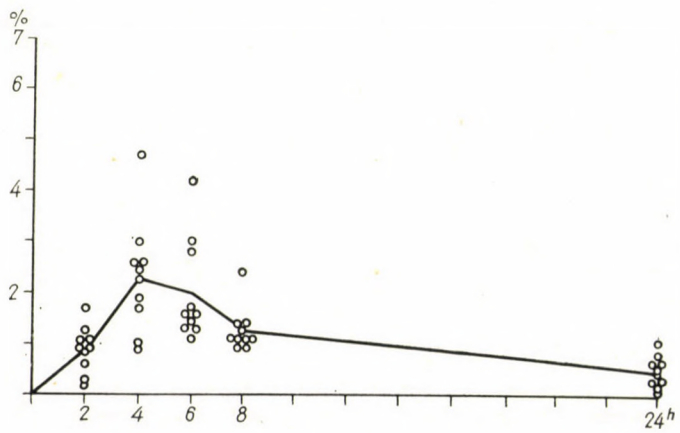

Abb. 2. Trichloressigsäurepräzipitat-Aktivitätswerte und Durchschnittskurve normaler Personen

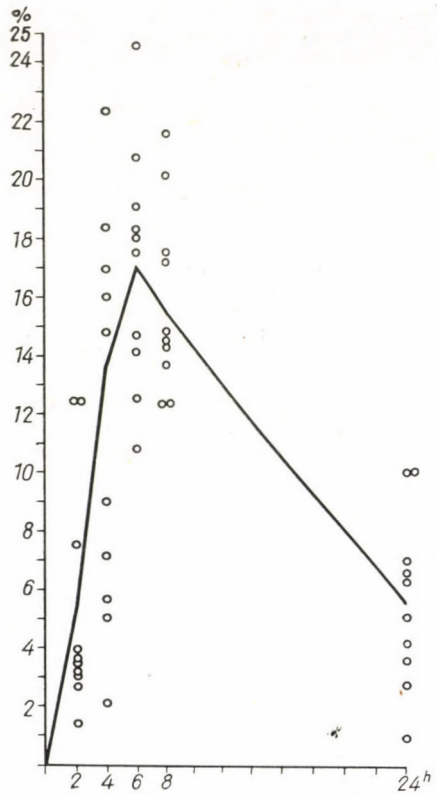

Abb. 3. Vollblutaktivitätswerte und Durchschnittskurve der Atherosklerotiker

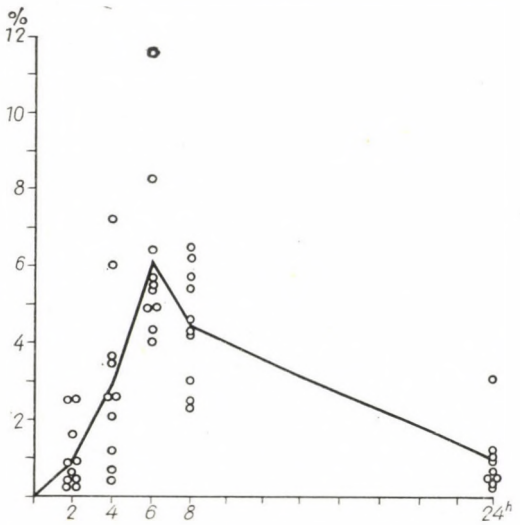

Abb. 4. Trichloressigsäurepräzipitat-Aktivitätswerte und Durchschnittskurve der Atherosklerotiker

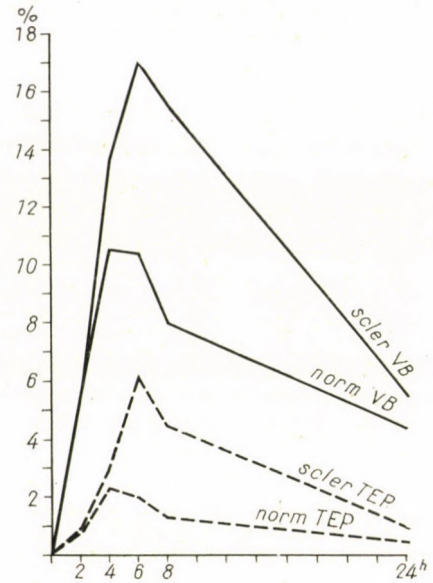

Abb. 5. Vollblut (VB)- und Trichloressigsäurepräzip. (TEP)-Durchschnittskurve bei Normalen und Atherosklerotikern

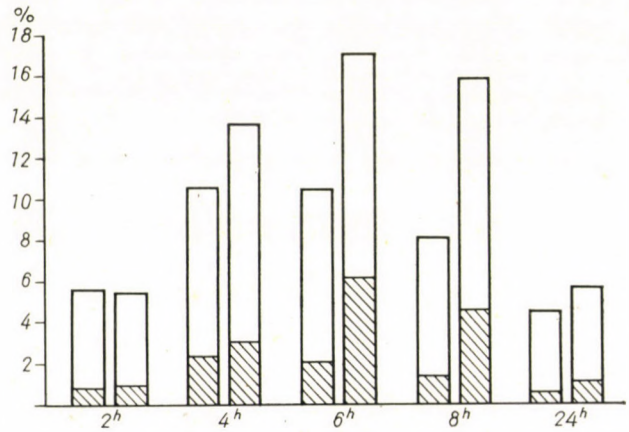

Abb. 6. 2, 4, 6, 8 und 24stündigen Durchschnittswerte bei Normalen und Atherosklerotikern. Erste Säule: Gesunde Personen, zweite Säule: Atherosklerotiker. Ganze Säule: Vollblutwerte, schraffierter Teil: Trichloressigsäurepräzipitat-Aktivität

sich wieder. Werden die Durchschnitte der Maximalwerte verglichen, so ist die Abweichung zwischen den beiden Gruppen am augenfälligsten (Abb. 7): Während der Durchschnitt der normalen Maximalwerte 11,45 ± 2,25% bzw. 2,64 ± 1,11% beträgt, gestalten sich die Ergebnisse der Atherosklerotiker: 18,1 ± 3,12% bzw. 6,5 ± 2,07% (p% < 0,1 bzw. p% < 0,1).

Die in Abb. 8 und 9 dargestellten Werte der Gruppe III (auf Atherosklerose verdächtige Patienten) lagen teils den Werten der Kontrollgruppe, teils den Ergebnissen der atherosklerotischen Gruppe näher. Infolge dieser Verteilung und der geringen Zahl der Fälle, wurden keine Durchschnittswerte berechnet.

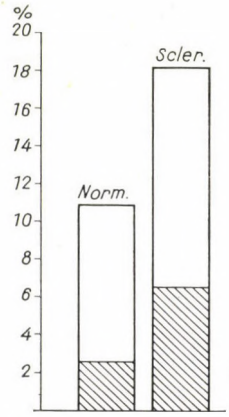

Abb. 7. Durchschnitte der Maximalwerte. Bezeichnung s. Abb. 6.

Tabelle I

Nr.	Benennung	Durchschnitte und Standarddeviationen				
		2 St	4 St	6 St	8 St	24 St
I.	Normalblut	5,53±1,21	10,53±2,09	10,38±2,22	8,03±2,39	4,53±1,65
II.	Blut der Atherosklerotiker	5,35±4,02	11,72±6,79	17,04±4,13	15,82±3,16	5,66±2,93
III.	Norm. Trichloressigs. Präzip.	0,85±0,42	2,32±1,09	2,03±0,98	1,31±0,42	0,46±0,25
IV.	Trichloressigs. Präzip. bei Atherosklerotikern	1,04±0,86	2,99±2,20	6,12±2,33	4,47±1,50	0,97±0,86
	I—II. p%	>80	>60	<0,1	<0,1	>30
	III—IV. p%	>50	>60	<0,1	<0,1	>10

2, 4, 6, 8 und 24 stündige Durchschnittswerte des normalen und atherosklerotischen Blutes bzw. der Trichloressigsäurepräzip. — Aktivität im Prozentsatz des verabreichten J^{131}-Trioleins ausgedrückt [mit Darstellung der Standarddeviationen]. Die zwei unteren Zeilen zeigen die Signifikanz zwischen dem normalen und atherosklerotischen Blut bzw. der Trichloressigsäurepräzip. — Aktivität in p%.

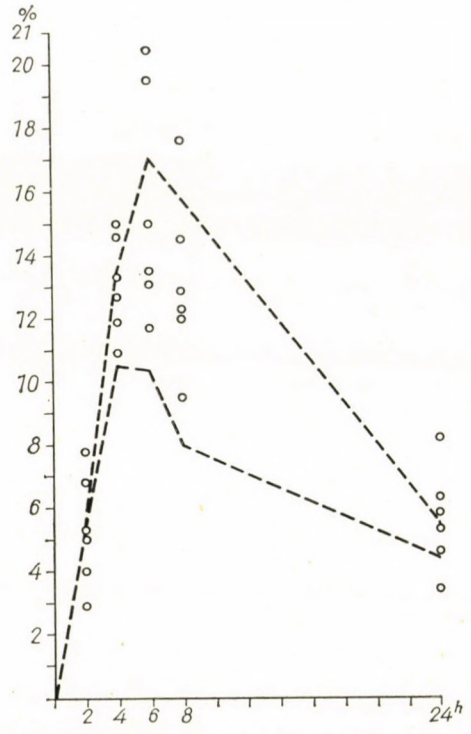

Abb. 8. Vollblutwerte bei Personen mit Atherosklerose-Verdacht. Obere und untere Strichellinie zeigt die Durchschnittskurve vom atherosklerotischen bzw. normalen Blut

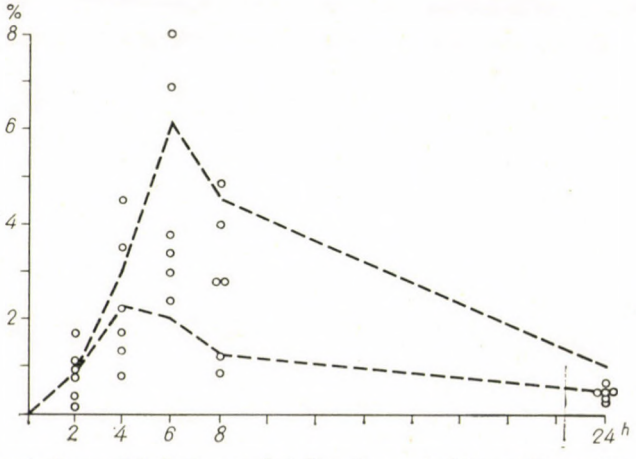

Abb. 9. Trichloressigsäurepräzipitatwerte bei Kranken mit Atherosklerose-Verdacht. Strichellinie s. Abb. 8

Besprechung

Die Anwendung des J^{131}-Trioleins bedeutete einen wesentlichen Fortschritt in der Untersuchung des Fettstoffwechsels. Obwohl nach VAN HANDEL und ZILVERSMITH [36, 37] der physiologische Stoffwechsel des Trioleins durch die Markierung modifiziert wird, halten die meisten Verfasser die Methode für diesbezügliche Untersuchungen geeignet [38—41]. Werden jedoch eindeutige Resultate erhalten, so ist die Methodik zu diagnostischen Vergleichsuntersuchungen auch in dem Fall geeignet, wenn der erwähnte Vorbedacht von VAN HANDEL und ZILVERSMITH akzeptiert wird.

Die J^{131}-Triolein-Monochlorid Bindung ist beständig gegen pH-Veränderungen von 1 bis 8, als auch gegen die Wirkung von 10%iger HCl, von Magen- und Duodenalsaft bzw. Pankreatin oder Pepsin [42]. Mehr als 90% des verabreichten J^{131}-Trioleins wird resorbiert [8]. Die im Vollblut gefundene Aktivität wird teilweise durch das an Lipoprotein gebundene, teilweise durch das während der Utilisation freigesetzte anorganische J^{131} geliefert. Da die Formelemente des Blutes am J^{131} Transport nicht beteiligt sind, können die Bestimmungen auch im Plasma durchgeführt werden [41]. Nach der Untersuchungen von BERES und Mitarb. [8] enthält der Trichloressigsäurepräzipitat ausschließlich organisch gebundenes, die Supernatans nur anorganisches J^{131}. Außerdem wurde bewiesen, daß die Radioaktivität des Präzipitats von der Lipoidfraktion der Lipoproteine stammt. LAKSHMINARAYANA und Mitarb. [40] haben nachgewiesen, daß sich das an Triolein gebunden verabreichte J^{131} im Blut in den Triglyzeriden befindet, d. h. die markierte Ölsäure wird als Triglyzerid transportiert, und das J^{131} wird weder in die Cholesterinester noch in das Phospholipid eingebaut.

Unsere Untersuchungsergebnisse bestätigen die Beobachtungen von LIKOFF und SELLER: Die J^{131}-Aktivität war im Vollblut und in der Trichloressigsäurefraktion der Atherosklerotiker im Verhältnis zu den Kontrollwerten signifikant höher bzw. verzögerter. Beachtungswert ist die Bewertung der dritten Gruppe, wo das Fehlen der auf Atherosklerose weisenden objektiven Befunde bei positiver Anamnese ein diagnostisches Problem bedeutete. Hinsichtlich des Alters bilden diese Patienten einen Übergang zwischen den beiden Gruppen. Aus den Untersuchungen von BERKOWITZ [43] geht jedoch hervor, daß der Verlauf der Aktivitätskurve durch das Alter allein nicht beeinflußt wird. Die Tatsache, daß die Aktivitätswerte teils den Normalwerten, teils den Werten der Atherosklerotiker naheliegen, kann vielleicht damit erklärt werden, daß ein Teil der Patienten an einem Frühstadium der Atherosklerose leidet bzw. ein Kandidat für Atherosklerose sei. Natürlich könnte diese Folgerung nur auf Grund von Untersuchungen an größeren Material und langere Beobachtungszeit gestellt werden.

Die Ursache des Unterschiedes, der zwischen dem J^{131}-Triolein Stoffwechsel der Atherosklerotiker und der gesunden Personen vorliegt, ist heute noch unbekannt. Es ist unwahrscheinlich, daß die Resorption verschieden wäre, da die Kurven in den ersten vier Stunden fast identisch sind. Es ist anzunehmen, das die Elimination bzw. Utilisation der Lipoide verändert sind. MENG [29], ferner CROFFORD und Mitarb. [27] bewiesen, daß Kranken mit essentieller Hyperlipämie nur in geringerem Maße fähig sind, die komplexen Triglyzeride in eine, für die Gewebszellen nützliche Form umzugestalten. Annehmbar verursacht auch bei den Atherosklerotikern ein ähnlicher Mechanismus — irgendeine Störung des Lipoidstoffwechsels — die Verzögerung der Elimination. Nach der Hypothese von GEORG und Mitarb. [44] besteht die nach J^{131}-Triolein-Resorption im Blut nachweisbare Lipoidaktivität aus 2 Fraktionen: Eine sich langsam eliminierende alpha-Fraktion zu ungefähr 5%, und eine sich rasch eliminierende beta-Fraktion zu etwa 95%. Es wird angenommen, daß im Blut der Kranken mit Koronarokklusion und Hyperlipämie die sich langsam eliminierende alpha-Fraktion zu Lasten der beta-Fraktion zunehmen würde.

Das abweichende Verhalten der Aktivitätskurve von Atherosklerotikern ist heute eine noch offene Frage, deren Klärung zur Kenntnis der Pathogenese der Erkrankung beitragen würde.

*

Das Triolein wurde von Herren Dr. M. JÁKI, Direktor des Forschungsinstituts der Chemischer Industrie für Pflanzenöle, und Abteilungsleiter Ing. chem. J. BERÉDI hergestellt. Die Markierung verfertigte Herr Ing. chem. E. VÁNDOR, wissenschaftlicher Mitarbeiter der Fabrik Reanal für Feinkemikalien. An dieser Stelle sei ihnen allen aufrichtig gedankt.

Die statistischen Berechnungen wurden vom Mathematischen Forschungsinstitut der Ungarischen Akademie der Wissenschaften ausgeführt.

LITERATUR

1. STANLEY, M. M., THANNHAUSER, S. J.: J. Lab. clin. Med. **34,** 1634 (1949).
2. BAYLIN, G. J., SANDERS, A. P., ISLEY, J. K., SHINGLETON, W. W., HYMANS, J. C., JOHNSTON, D. H., RUFFIN, J. M.: Proc. Soc. exp. Biol. (N. Y.) **89,** 54 (1955).
3. WELLS, M. H., SHINGLETON, W. W., SANDERS, A. P.: Proc. Soc. exp. Biol. (N. Y.) **90,** 717 (1955).
4. SHINGLETON, W. W., WELLS, M. H., BAYLIN, G. J., RUFFIN, J. M.: Surgery **38,** 134 (1955).
5. SHINGLETON, W. W., BAYLIN, G. J., ISLEY, J. K., SANDERS, A. P., RUFFIN, J. M.: Ann. Surgery **144,** 433 (1956).
6. RUFFIN, J. M., SHINGLETON, W. W., BAYLIN, G. J., HYMANS, J. C., ISLEY, J. K., SANDERS, A. P., SOHMER, M. F.: New Engl. J. Med. **255,** 594 (1956).
7. SHINGLETON, W. W., BAYLIN, G. J., ISLEY, J. K., SANDERS, A. P., RUFFIN, J. M.: Gastroenterology **32,** 28 (1957).
8. BERES, P., WENGER, J., KIRSNER, J. B.: Gastroenterology **32,** 1 (1957).
9. BERKOWITZ, D., SKLAROFF, D.: Arch. intern. Med. **100,** 951 (1957).
10. MCKENNA, R. D., BOURNE, R. H., MATZKO, A.: Gastroenterology **32,** 17 (1957).

11. DUFFY, B. J., TURNED, D. A.: Ann. intern. Med. **48,** 1 (1958).
12. GROSSMAN, M. I., JORDAN, P. H.: Gastroenterology **34,** 901 (1958).
13. ISLEY, J. K., SANDERS, A. P., BAYLIN, G. J., RUFFIN, J. M., SHINGLETON, W. W., ANLYAN, W. G., SHARPE, K. W.: Gastroenterology **35,** 482 (1958).
14. KAPLAN, E., EDIDIN, B. D., FRIN, R. C., BAKER, L. A.: Gastroenterology **34,** 901 (1958).
15. RUFFIN, J. M., KEEVER, J. C., CHEARS, C. JR., SHINGLETON, W. W.: Gastroenterology **34** 484 (1958).
16. BERKOWITZ, D., SKLAROFF, D., WOLDOW, A., JAKOBS, M. G., LIKOFF, W.: Ann. intern. Med. **50,** 247 (1959).
17. GERTICH, I., BERNDT, H., ERNST, H.: Acta biol. med. germ. **3,** 188 (1959).
18. MOHAMED, S. D., HUME, R.: J. clin. Path. **12,** 535 (1959).
19. GRANDONICO, F., SALVINI, L., CITI, S.: Minerva gastroent. **6,** 1 (1960).
20. HENNING, N., SCHÖN, H., FAHSOLD, W.: Dtsch. med. Wschr. **85,** 777 (1960).
21. REITH, W. S., WILLIAMS, E. S., THOMAS, M. J.: Lancet **2,** 1229 (1961).
22. LIKOFF, W., BERKOWITZ, D., WOLDOW, A., JAKOBS, G.: Circulation **16,** 908 (1957).
23. LIKOFF, W., BERKOWITZ, D., WOLDOW, A., JAKOBS, G., SKLAROFF, D.: Circulation **18,** 1118 (1958).
24. SELLER, R. H., BRACHFELD, J., SANDBERG, H., BELLET, S.: Amer. J. Med. **27,** 231 (1959)
25. SANDBERG, H., MIN, B. S., FEINBERG, L., BELLET, S.: Arch. intern. Med. **105,** 866 (1960).
26. HAKKILA, J., MÄKELÄ, T. E., HALONEN, P. I.: Amer. J. Cardiol. **5,** 295 (1960).
27. CROFFORD, O. B., CONIGLIO, J. G., MENG, H. C., NEWMAN, E. W.: J. clin. Invest. **37,** 886 (1956).
28. SIGLER, M. H., RUBINI, M. E.: Arch. intern. Med. **107,** 894 (1961).
29. MENG, H. C.: Amer. J. clin. Nutr. **9,** 68 (1961).
30. METZ, J., ANTONIC, A., BERSOHN, I., HART, D.: Brit. med. J.: 1270 1960/II.
31. SCHWARTZ, M. J., LEWITUS, Z.: J. Atheroscler. Res. **2,** 68 (1962).
32. BERKOWITZ, D., LIKOFF, W., SKLAROFF, D.: Amer. J. Cardiol. **4,** 282 (1959).
33. DUTMINI, F. M., SARDI, A.: Drugs Affecting Lipid Metabolism. Elsevier, Amsterdam 1961.
34. EDELMAN, J., KERSHBAUM, A., SANDBERG, H., BELLET, S.: Amer. J. med. Sci. **241,** 138 (1961).
35. SAMUEL, P., WAITHE, W. I.: Circulation **24,** 578 (1961).
36. VAN HANDEL, E., ZILVERSMITH, D. B.: J. Lab. clin. Med. **52,** 831 (1958).
37. VAN HANDEL, E., ZILVERSMITH, D. B.: Fed. Proc. **16,** 131 (1957).
38. KRUGER, F. A., CORNWELL, D. G., HAMWI, G. J., BROWN, J. B.: Fed. Proc. **17,** 258 (1958).
39. KRUGER, F. A., CORNWELL, D. G., HAMWI, G. J., BROWN, J. B.: Clin. Nutr. **8,** 44 (1960).
40. LAKSHMINARAYANA, W. H., KRUGER, F. A., CORNWELL, D. G.: Fed. Proc. **18,** 269 (1959).
41. TURNER, D. A.: Amer. J. dig. Dis. **3,** 594 (1958).
42. BÁLINT, J., PENDOWER, J., RAMSEY, N. W.: Clin. Sci. **19,** 321 (1960).
43. BERKOWITZ, D., SKLAROFF, D., LIKOFF, W.: J. Amer. Geriat. Soc. **7,** 741 (1959).
44. GEORGE, E. P., FARKAS, G. S., SOLLOCH, W.: J. Lab. clin. Med. **57,** 167 (1961).

Gábor ERDÉLYI
László KELLER
Imre BALÁZSI
Budapest VIII., Mező I. u. 15—17. Ungarn.

ACTA MEDICA

ТОМ XIX – ВЫП. 1

РЕЗЮМЕ

ИЗМЕНЕНИЯ ЭКГ И КРОВООБРАЩЕНИЯ У БОЛЬНЫХ, СТРАДАЮЩИХ ПРОГРЕССИРУЮЩЕЙ МЫШЕЧНОЙ ДИСТРОФИЕЙ

Ф. ШОЛЬТИ, Э. ЗАДОРИ и ДЬ. БЕКЕНЬ

В связи с 22 больными, страдающими прогрессирующей мышечной дистрофией, исследовались изменения ЭКГ и кровообращения, наступающие при этой болезни. Правильный диагноз был подтвержден, кроме клинических исследований, также биопсией мышц.

В отношении изменений кровообращения часто наблюдались низкий систолический объем и повышенное периферическое сопротивление. Часто было отмечено запаздывание механической систолы по сравнению с электрической систолой.

ЭКГ показала свыше чем в 4/5 части случаев патологические изменения, которые можно отнести в три основных группы.

Обсуждается механизм возникновения изменений ЭКГ и кровообращения.

РАССТРОЙСТВА ЭЛЕКТРОЛИТНОГО И ВОДНОГО ОБМЕНА, ВЫЗВАННЫЕ ОБЛУЧЕНИЕМ, С ОСОБЫМ УЧЕТОМ ИЗМЕНЕНИЙ КОЛИЧЕСТВА ИОНОВ ВО ВНЕКЛЕТОЧНОМ ПРОСТРАНСТВЕ

З. ЖЕБЁК, ДЬ. ПЕТРАНЬИ МЛ. и И. ВАХТЛ

Авторы дают краткий литературный обзор о расстройствах электролитного и водного обмена живых существ, в частности млекопитающих, пораженных лучами. Исследовалось изменение содержания общего Na и K во внеклеточном пространстве при 3—5 дневном эффекте, что бросает свет на скрытые изменения содержания ионов в организме. Зарегистрировались также изменения количества ионов, выделяемых мочой. Полученные экспериментальные данные обсуждаются в свете воззрения, формирующегося в настоящее время по этому вопросу.

ИЗМЕНЕНИЯ ЭКГ, СВЯЗАННЫЕ С ОПОЯСЫВАЮЩИМ ЛИШАЕМ

И. ПАШТИНСКИ и И. КЕНЕДИ

Авторы при серийных исследованиях наблюдали изменения ЭКГ у 26 больных с опоясывающим лишаем, охватившим главным образом грудные сегменты. Изменения ЭКГ отмечались преимущественно у молодых мужчин (53,8%), причем изменения ухудшались или улучшались параллельно течению процесса опоясывающего лишая. В тяжелых случаях заболевания опоясывающим лишаем, поражающим несколько сегментов, изменения ЭКГ наблюдались значительно чаще, чем в простых случаях. Обращается внимание на то, что при опоясывающем лишае может возникать патологический глубокий зубец Q, который сохраняется без кардиальных симптомов. Изменения ЭКГ чаще всего отмечаются в случае опоясывающего лишая, поражающего сегменты D_{2-7}.

Кардиальный эффект опоясывающего лишая (cardiopathia zosterica) может возникать двумя способами: 1) вследствие нарушения симпатической иннервации, вызванного ганглионитом и 2) васкулярным путем, вследствие гиперергического сосудистого заболевания пораженного сегмента, наподобие arteritis (Feyrter). Для последнего предположения в отношении сердца еще не имеются гистологических доказательств.

ЭКСПЕРИМЕНТАЛЬНОЕ ИССЛЕДОВАНИЕ ЭФФЕКТА СТРОФАНТИНА НА ФУНКЦИЮ ПОЧЕЧК

Ф. ШОЛЬТИ, И. МАРТОН, Ю. РЕВ и Р. ХЕРМАНН

Исследовалось действие строфантина на функцию почек и механизм этого действия на больных с нормальным кровообращением и с хронической сосудистой недостаточностью и в опытах на собаках. Было установлено, что при остром действии строфантина значительно повышается выделение воды и калия, и в меньшей мере — натрия. Указанный эффект наступает как у лиц с нормальным кровообращением, так и у больных с хронической недостаточностью кровообращения. Вызванное строфантином повышение выделения соли и воды основывается прежде всего на снижении канальцевой резорбции, и его нельзя отнести к изменению кровообращения. Уровень АДГ в сыворотке не показывает достоверного изменения после дачи строфантина. При наркозе баитиналом вышеописанное действие строфантина на почки не наступает. Предварительная дача симпатико-адренолитического дибенамина или предварительная перерезка симпатических нервов, идущих к почкам, так-же предотвращают обусловленное строфантином повышение выделения соли и воды. Введение строфантина в голову, после полной изоляции ее от кровообращения туловища, вызывало типичное повышение выделения воды и соли.

По результатам опытов строфантин действует на почки прежде всего через центральную нервную систему, и через симпатические нервные пути, идущие к почкам.

ЛЕЧЕНИЕ АНТИКОАГУЛЯНТАМИ ТРОМБОЗОВ И ЭМБОЛИЙ АРТЕРИЙ КОНЕЧНОСТЕЙ

К. БУГАР-МЕСАРОШ и Й. ФОНО

Авторы в 56 случаях тромбоза и в 40 случаях эмболии артерий конечностей применяли лечение антикоагулянтами, преимущественно препаратами кумарина. 20 больных получили также гепарин внутриартериально, некоторые из них в начале лечения, внутривенно. В качестве контроля служило 22 больных с артериальным тромбозом и 40 больных с эмболией.

В группе больных, леченных антикоагулянтами, терапевтический эффект был лучшим, чем в контрольной группе. Из 96 больных первой группы у 70 отмечалось значительное улучшение, у 13 — улучшение небольшой степени, у 7 — состояние не изменилось, в то время как у 6 больных отмечалось ухудшение состояния. Во второй группе из 62 больных значительное улучшение отмечалось только у 13, умеренное улучшение у 28, состояние неизменилось у 3, а ухудшение зарегистрировано у 18 больных. Особенно выраженная разница наблюдается между двумя группами в случаях эмболии конечностей в пределах 24 часов, так как у 18 больных группы, получившей антикоагулянты, ни в одном случае не пришлось провести ампутацию конечности, в то время как в контрольной группе среди 21 больного у 6 не удалось спасти конечность.

На основании благоприятных результатов лечения авторы считают, что в случаях артериальных тромбозов и в не поддающихся эмболэктомии случаях эмболий конечностей — при отсутствии противопоказаний — лечение антикоагулянтами можно рассматривать вполне обоснованным.

ЭФФЕКТ ХЛОРПРОПАМИДА НА ГЛИКОНЕОГЕНЕЗ В ПЕЧЕНИ

А. КАЛЬДОР и Г. ПОГАЧА

Авторы выявили, что наблюдаемое на препаратах при перфузии аланина и пировиноградной кислоты снижение отдачи сахара может быть предотвращено хлорпропамидом. Подобное действие нельзя вызвать гликолом. В случае перфузии пировиноградной кислоты хлорпропамид задерживает также снижение уровня гликогена в печени.

ЭФФЕКТ КОКАРБОКСИЛАЗЫ НА МИНУТНЫЙ ОБЪЕМ ПРИ ОСТРОЙ ИНДУЦИРОВАННОЙ ГИПОКСИИ

З. НАДЬ и И. ШКОЛЬНИК

В экспериментах на собаках повышение минутного объема, вызванное острой артериальной гипоксией, можно уменьшить кокарбоксилазой, несмотря на то, что степень артериальной десатурации совершенно не изменяется. При нормальной сатурации кислородом ТРР не оказывает никакого влияния на минутный объем.

ГУМОРАЛЬНОЕ РЕГУЛИРОВАНИЕ ТРОМБОЦИТОПОЭЗА

К. РАК

Подытоживаются наблюдения, имеющиеся относительно этого сывороточного фактора белковой природы, и излагается метод его выявления как и клинический материал, охватывающий 139 больных. В мышином тесте вызывающее тромбоцитоз свойство сыворотки с наибольшей частотой можно выявить при полицитемии, гиперсплении и острой тромбоцитопении. При введении больным с тромбоцитопенией нормальная кровь или плазма также могут иметь тромбопоэтический эффект. Вкратце обсуждается вопрос возникновения этого фактора, его способа действия и патогенетическая роль. Можно установить отклонения при сравнении с эритропоэтином.

ИЗМЕНЕНИЯ ЭЭГ, ВЫЗВАННЫЕ ЭЛЕКТРОШОКОВОЙ НАГРУЗКОЙ У ШИЗОФРЕНИКОВ

И. ХУСАК и К. ШОМОДЬИ

На основании электроэнцефалограмм, регистрированных у шизофреников и нешизофреников в течение первичного лечения электрошоком можно установить нижеследующее:

1) В контрольных случаях нормализация электроэнцефалограммы после шока наступала в пределах 10—15 мин.

2) У шизофреников для процесса нормализации требовалось 35—40 мин. и даже больше; у контрольных больных нормализация наступает крутой прямой линией.

3) На основании затяжности нормализации и интермиттирующих возвратов можно заключить, что у шизофреников функциональная способность процессов обмена веществ головного мозга, поддерживающих функционирующие структуры, нарушена.

ИССЛЕДОВАНИЕ БОЛЬНЫХ АТЕРОСКЛЕРОЗОМ ПРИ ПОМОЩИ ТРИОЛЕНА, МЕЧЕНОГО J^{131}

Г. ЕРДЕИ, Л. КЕЛЛЕР и И. БАЛАЖ

Авторы установили, что в цельной крови и в трихлоруксуснокислотном осадке крови больных атеросклерозом кривая активности введенного через рот триолеин монохлорида, меченого J^{131}, оказывается более затяжной и более высокой, чем у здоровых контрольных лиц. У больных с подозрением на коронарный склероз, у которых обычными клиническими методами исследования не удалось выявить изменений, величины активности были отчасти близки к средним значениям контрольной группы, а отчасти к средним данным группы атеросклеротиков. На основе литературных данных, как и полученных до сих пор собственных результатов, авторы придерживаются того мнения, что этот способ может оказать помощь при постановке раннего диагноза атеросклероза.


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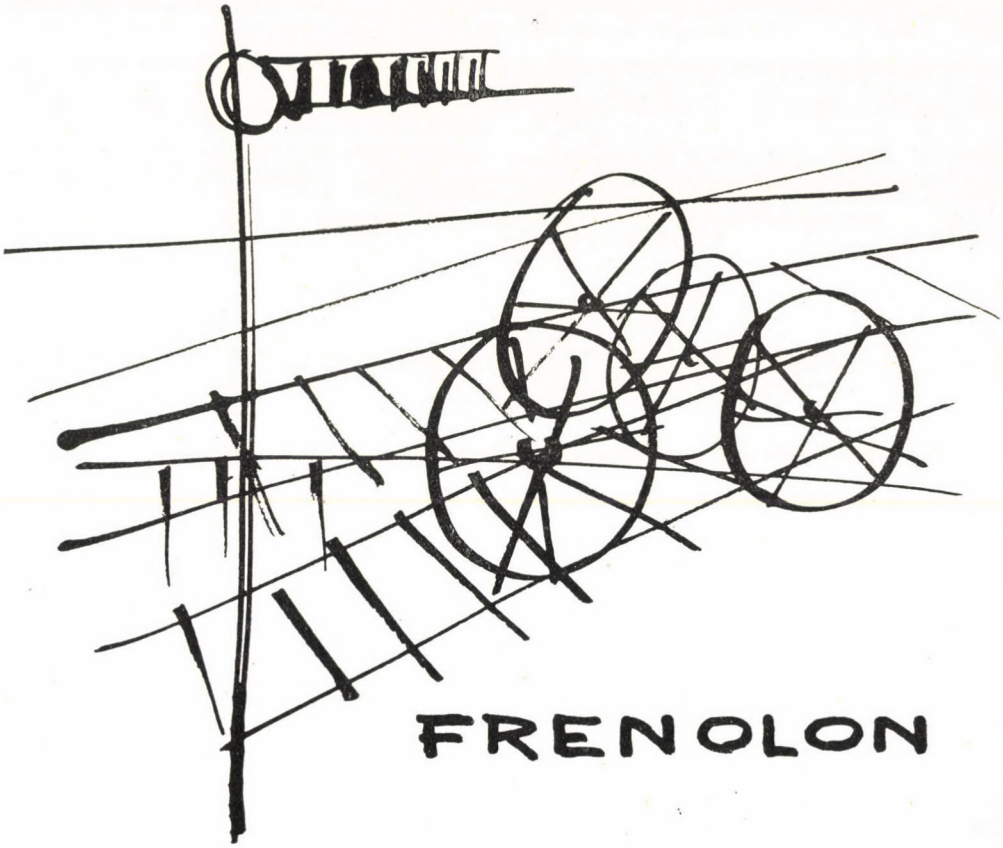

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A kézirat nyomdába érkezett: 1963. I. 2. — Terjedelem: 8,50 (A/5) ív, 44 ábra

63.56464 — Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György

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Index: 26.015

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MECHANISM OF THE "REGRESSION" OF DIABETES INSIPIDUS AFTER PITUITARY STALK LESIONS IN RATS

By

K. KOVÁCS, MARGIT A. DÁVID and F. A. LÁSZLÓ

FIRST DEPARTMENT OF MEDICINE, UNIVERSITY MEDICAL SCHOOL, SZEGED

(Received August 2, 1962)

The water metabolism of rats has been studied several weeks after the pituitary stalk had been destroyed by means of the Horsley—Clarke apparatus.

It has been found that on an oral load of tap water the polyuric reaction systematically observed after the operation ceased gradually in most of the animals, and 1 to 2 months after operation diuresis did not exceed, or was only slightly more than the average for the non-operated control rats. The administration of ethanol caused moderate, that of physiological saline or cortisone intensive polyuria. Exogenous creatinine clearance was decreased, and so were the specific gravity and electrolyte content of the urine. However, the cessation of the diabetes insipidus-like condition was only virtual, because spontaneous water intake was still increased several weeks after operation. Antidiuretic activity was significantly reduced in the hypothalamus and hardly demonstrable in the neurohypophysis. Morphologically, significant atrophy and the disappearance of the neurosecretory material were observed.

The evidence obtained indicates that the production of antidiuretic hormone is not resumed several weeks after the destruction of the pituitary stalk, and thus this hormone has no significant role to play in the regression of the polyuric reaction. The cessation of polyuria can be traced back first of all to a diminution of glomerular filtration; involvement of other factors cannot be ruled out, either. The changes in renal circulation may result partly from the pituitary-adrenocortical hypofunction caused by the destruction of the stalk.

In previous experiments concerned with the hypothalamic-hypophysial relations of water metabolism [21] it was found that in rats with stalk lesion, obviously as a result of damage to the tissues producing the antidiuretic hormone, the oral administration of tap water was followed by considerable polyuria. However, when water loads were applied through longer periods, diuresis gradually diminished after a few weeks in most animals and one or two months after operation it did not exceed, or hardly exceeded, the mean values obtained in the non-operated control animals. The results tend to indicate that in most of the rats destruction of the pituitary stalk failed to produce persisting diabetes insipidus.

Other authors, too, found that in the rat operations around the hypophysial area may be followed by a regression of diabetes insipidus. For example, it has been shown by CHESTER JONES [7] that spontaneous water intake was merely temporarily increased in some of the neurohypophysectomized rats. ALEXANDER [1] lesioned the hypothalamus in several hundred rats and observed that the polyuria following operation was not always permanent; chronic

diabetes insipidus developed in merely one-third of the animals showing considerable polyuria during the period immediately following operation. There is ample evidence [4, 6, 16, 17, 18] to indicate that in animals subjected to total hypophysectomy transitory polyuria, but no lasting diabetes insipidus, develops.

In the light of these data it seemed interesting to study on the one hand the water metabolism of rats stalk-lesioned several weeks before by methods other than that involving tap water loads and on the other, to investigate what mechanisms might play a role in the regression of the polyuric reaction observable in response to oral tap water administration during the postoperative period.

In the present paper the results obtained in the above experiments are described.

Materials and methods

Albino rats of either sex, fed at mixed diet and weighing 140 to 220 g, were used. The pituitary stalk was lesioned under hexobarbital anaesthesia, by means of the Horsley—Clarke apparatus. Water metabolism was controlled several times after operation. In the experiments to be described only those rats were used which showed polyuria in response to the oral administration of tap water in the postoperative period, or in which the polyuria ceased one month after destroying the stalk and diuresis no longer differed significantly from the average for the non-operated controls. These two criteria were supplemented by a third one, *viz.* the hypothalamic-hypophysial system of the animals was studied histologically, in serial sections. Only those animals were taken into consideration as to the changes in water metabolism, in which the hypophysial stalk had been destroyed in its entire length (so-called total stalk lesion).

The animals not meeting the above criteria, *viz.* those in which the stalk had not or only partially been damaged or in which no disturbance of water metabolism had developed after operation, were not included in the evaluation. (It has to be noted that total stalk lesion invariably resulted in polyuria.) In a few rats with total stalk lesion the diabetes insipidus-like condition did not cease after 2 or 3 months; these animals continued to respond with polyuria to the oral administration of tap water. Since our aim was to study the regression of diabetes insipidus, these animals, too, were excluded from the evaluation.

The technique of water load and the details of the histological examination of the hypothalamic-hypophysial area, the localization of the lesion and determination of its extent in our previous papers [15, 16, 17, 21] have been described. The diuretic reaction of the animals was evaluated as a "summation urine output" after the oral administration of 5 per cent body weight of tap water. This value indicates the intensity of diuresis and is the area under the summation polygon of the cumulative urine outputs measured at 1-hour intervals for 8 hours multiplied by the reciprocal of the amount of water administered. The specimens for histologic study were obtained in the following way. The rats were killed by decapitation, the hypothalamus was removed together with the pituitary (thus it was achieved that the stalk could be examined as a whole), fixed in Susa's solution, embedded in paraffin and cut up serially into 12-micron thick sections at 100 micron distances. The sections cut in the frontal plane were stained with haematoxylin-eosin, in some cases with Gomori's chrome-alum-haematoxylin, Gomori's aldehyde-fuchsin, as well as with Paget-Eccleston's aldehyde thionine.

We studied several aspects of the water metabolism of rats stalk-lesioned more than one month earlier. Following the oral administration of tap water we determined the electrolyte and creatinine excretion, the specific gravity of the urine, the glomerular filtration rate, spontaneous water intake, the histology of the hypothalamus and neurohypophysis, as well as the antidiuretic principle they contained. Finally, we made some experiments to influence water metabolism. The results obtained were compared with those yielded by non-operated controls, or, if it seemed necessary, with those obtained in rats with stalk-lesions a few days before and being polyuric.

Urinary excretion of sodium, potassium, chloride, and creatinine

The animals had been fasted for 10 hr. before the experiment, tap water had been allowed ad libitum. At the beginning of the experiment water was withheld, the animals were placed one by one into cages suitable for collecting urine, and were given by stomach tube tap water or physiological NaCl solution, in amounts of 5 per cent body weight. The duration of the experiment was 5 hours. By the end of the 5th hour urine volume was measured with 0.1 ml precision, and samples of urine were tested for Na, K, Cl and creatinine concentration. Na and K were determined by flame photometry, Cl by the method of SCHALES and SCHALES [32], creatinine by the method of FOLIN-WU [11], as modified by BROD and SIROTA [5].

The volume of urine excreted in 5 hr. was computed for 100 g. body weight. The values of sodium, potassium and chloride are expressed in m-Eq./l., as well as in μ-Eq./100 g. body weight. The creatinine values are expressed in mg. creatinine output in 5 hr., computed for 100 g. body weight. Finally the ratio of sodium and potassium (Na/K) was also estimated. The results were statistically analysed using Student's t-test.

The results for electrolyte and creatinine excretion are presented in *Table I*. The average urine output of the rats with stalk lesions given tap water did not substantially differ from that of the non-operated controls. However, as compared corresponding control values, urinary Na, K, Cl concentration and output were significantly lower. The Na/K ratio did not change, whereas creatinine excretion slightly decreased. In *Table I* there are data also for 10 rats which had been subjected to stalk lesion a few days earlier. These animals voided significantly more urine. The concentrations of Na, K and Cl decreased, total electrolyte output was practically unchanged, creatinine output was slightly increased.

The rats subjected to stalk lesion several weeks earlier responded to the oral administration of physiological saline solution with marked polyuria. The values are almost identical with, or slightly different from, those for the rats stalk-lesioned a few days earlier. Urine output increased in both groups, Na, K and Cl concentrations decreased, K and creatinine outputs were practically unchanged, whereas Na and Cl excretion was slightly increased. Comparison of the data for the tap water-treated animals with those for the physiological saline-treated ones shows that following the administration of saline the output not only of Na and Cl, but also of K increased. It is noteworthy that the hyperpotassuria developed in the stalk-lesioned animals, too. According to the data of BRUNNER et al. [6] and our own [16, 18] urinary K concentration does not increase in hypophysectomized rats treated orally with physiological NaCl solution. Thus, our results suggest that as far as potassium excretion

Table I

Sodium, potassium, chloride and creatinine outputs following oral administration of tap

	Group	Time after operation	Fluid administered	Number of animals	Body weight g	5 hr urine output calculated for 100 g body weight (ml)	Na
I.	Intact	—	tap water	10	162.5 ±2.3*	4.9 ±0.1	29.8 ±4.4
II.	Stalk-lesions	6—8 days	tap water	10	191.5 ±7.9	9.6 ±0.6	15.2 ±1.1
III.	Stalk-lesions	more than 1 month	tap water	10	182.5 ±5.0	4.7 ±0.2	13.5 ±2.0
IV.	Intact	—	phys. NaCl	10	176.0 ±2.7	3.9 ±0.2	151.9 ±4.5
V.	Stalk-lesions	6—8 days	phys. NaCl	10	208.0 ±9.9	10.9 ±0.5	70.4 ±6.7
VI.	Stalk-lesions	more than 1 month	phys. NaCl	10	186.0 ±4.6	9.9 ±0.7	82.1 ±8.5

* Standard error.

Probability:		
I/II.	$p \ll 0.001$	$0.02 > p > 0.01$
I/III.	$p > 0.05$	$p < 0.01$
I/IV.	$p < 0.01$	$p \ll 0.001$
II/III.	$p < 0.001$	$p > 0.05$
II/V.	$p > 0.05$	$p \ll 0.001$
III/IV.	$p > 0.05$	$p \ll 0.001$
IV/V.	$p \ll 0.001$	$p < 0.001$
IV/VI.	$p < 0.001$	$p \ll 0.001$
V/VI.	$p > 0.05$	$p > 0.05$

is concerned, stalk-lesioned animals react like the intact ones, and not like hypophysectomized ones.

Specific gravity of the urine

The specific gravity of urine was determined by the pyknometric method of GÁL et al. [12], simultaneously with urine output 5 hours after the animals had been given 5 per cent body weight of tap water or physiological saline by mouth. The results are shown in *Fig.* 1. The rats loaded with physiological saline voided less urine of higher specific gravity than did the intact animals after water loading. A few days after lesioning the pituitary stalk both groups voided ample amounts of urine of lower specific gravity. There was no marked difference in specific gravity of the urine between intact and hypophysial

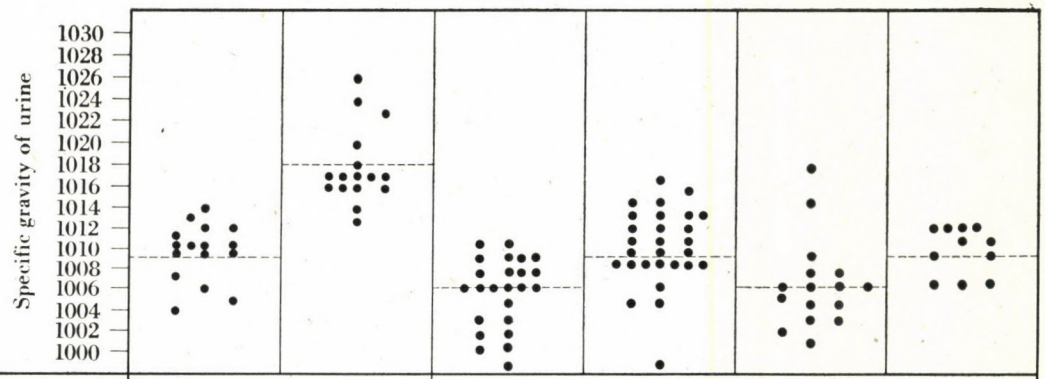
water and physiological NaCl solution, in intact rats and rats with stalk lesions

Concentration (mEq/l)		5 hr. output, calculated for 100 g body weight (μEq/l/100 g body weight)			Na/K ratio	5 hr. creatinine output calculated for 100 g body weight (mg)
K	Cl	Na	K	Cl		
14.8 ±2.3	32.6 ±5.5	148.1 ±22.5	69.4 ±11.2	163.8 ±27.6	2.1 ±0.2	0.44 ±0.03
7.0 ±0.4	16.5 ±2.1	145.3 ±18.7	66.0 ± 4.6	155.5 ±22.0	2.2 ±0.2	0.54 ±0.06
6.7 ±0.5	10.5 ±1.3	62.0 ± 8.8	31.9 ± 3.2	47.9 ± 5.4	1.8 ±0.3	0.34 ±0.04
27.2 ±2.2	184.2 ±5.6	585.6 ±33.5	105.4 ± 6.5	702.9 ±33.8	4.9 ±0.5	0.48 ±0.08
11.7 ±1.4	79.6 ±5.7	723.7 ±56.0	122.8 ±12.4	839.1 ±52.1	6.3 ±0.5	0.56 ±0.08
11.4 ±1.2	87.4 ±5.8	758.4 ±30.5	110.1 ±13.2	874.4 ±51.8	7.8 ±0.9	0.47 ±0.06
$p<0.01$	$0.02>p>0.01$	$p>0.05$	$p>0.05$	$p>0.05$	$p>0.05$	$p>0.05$
$p<0.01$	$p<0.001$	$p<0.01$	$p<0.01$	$p<0.001$	$p>0.05$	$p>0.05$
$p<0.01$	$p<<0.001$	$p<<0.001$	$0.02>p>0.01$	$p<<0.001$	$p<0.001$	$p>0.05$
$p>0.05$	$0.05>p>0.02$	$p<0.001$	$p<0.001$	$p<0.001$	$p>0.05$	$0.02>p>0.01$
$p<0.01$	$p<<0.001$	$p<<0.001$	$p<0.001$	$p<<0.001$	$p<0.001$	$p>0.05$
$p<<0.001$	$p<<0.001$	$p<<0.001$	$p<<0.001$	$p<<0.001$	$p<0.001$	$p>0.05$
$p<0.001$	$p<0.001$	$0.05>p>0.02$	$p>0.05$	$0.05>p>0.02$	$p>0.05$	$p>0.05$
$p<0.001$	$p<0.01$	$p<0.01$	$p>0.05$	$0.02>p>0.01$	$0.02>p>0.01$	$p>0.05$
$p>0.05$	$p>0.05$	$p>0.05$	$p>0.05$	$p>0.05$	$p>0.05$	$p>0.05$

stalk-lesioned rats after the water load. This was not surprising, since the non-operated animals had also been treated with tap water. There was more difference between the two groups on treatment with physiological saline. The specific gravity of the urine was low in the group subjected to stalk-lesion several weeks earlier after both water and saline load. Urine output by the animals treated with tap water was comparable to that of the non-operated controls, whereas in response to saline, intensive polyuria developed.

Glomerular filtration rate

Glomerular filtration rate was determined by the exogenous creatinine clearance method of LOTSPEICH [26]. Rats stalk-lesioned several weeks earlier and intact rats were used. One group of the stalk-lesioned animals was treated



Group	Intact		Hypophysial stalk-lesions			
Fluid administered	tap water	phys. NaCl	tap water	phys. NaCl	tap water	phys. NaCl
Time elapsed after operation	–	–	6 to 14 days	6 to 14 days	more than one month	more than one month
Number of animals	16	16	24	28	16	11
Body weight, g	190·0±3·7*	188·9±12·8	187·5±3·8	180·7±3·0	171·6±5·1	182·3±5·9
5 hr. urine output calculated for 100 g. body weight (ml)	4·3±0·1	2·2±0·5	8·9±0·5	9·5±0·3	4·3±0·4	8·4±0·4
Specific gravity of urine	1009±0·9	1018±0·8	1006±0·5	1009±0·4	1006±1·0	1009±0·5

* Standard error.

Fig. 1. Specific gravity of urine in intact rats and rats with stalk-lesions following oral loading with tap water and physiological NaCl solution

with subcutaneous doses of cortisone (Adreson, Organon, 10 mg/rat) over 4 days. The results are shown in *Table II*. Our data indicate that in this series diuresis and exogenous creatinine clearance rate significantly decreased in the animals stalk-lesioned several weeks before. These changes were abolished by cortisone treatment; in the animals stalk-lesioned several weeks before and so treated minute diuresis and exogenous creatinine clearance were comparable with those for the non-operated animals.

Table II

Exogenous creatinine clearance and minute diuresis in intact rats and rats with stalk-lesions

Group	Time after operation	Number of animals	Body weight g	Exogeneous creatinine clearance ml/100 cm²/min	Urine output ml/100 cm²/min
I. Intact	—	11	168.2 ± 8.9*	0.709 ± 0.076	0.030 ± 0.002
II. Stalk-lesions	more than 30 days	12	185.8 ± 5.7	0.438 ± 0.050	0.018 ± 0.002
III. Stalk-lesions + cortisone	more than 30 days	7	152.1 ± 3.2	0.703 ± 0.078	0.028 ± 0.002
		Probability:	I/II	$p<0.01$	$p<0.001$
			I/III	$p>0.05$	$p>0.05$
			II/III	$p<0.01$	$p<0.01$

* Standard error.

Water consumption

In an effort to study the nature of the disturbance of water metabolism observable in rats stalk-lesioned several weeks earlier, we have examined the 24-hour spontaneous water intake by non-operated control rats, and the rats stalk-lesioned 1 or 2 weeks earlier and responding with polyuria to oral water administration, comparing the results with those obtained for the rats stalk-lesioned several weeks before. The results are presented in *Table III*. They indicate that 1 or 2 weeks after the stalk had been lesioned water consumption significantly increased. However, water intake was increased also in the rats stalk-lesioned several weeks before, although such animals no longer developed polyuria on the water load.

Morphology of the hypothalamus and neurohypophysis

The hypothalamic-neurohypophysial system of the rats stalk-lesioned several weeks earlier showed characteristic morphological changes. The changes found in the adenohypophysis and the pars intermedia are not discussed here.

Table III

Spontaneous 24-hour water intake by intact rats and rats with stalk-lesions

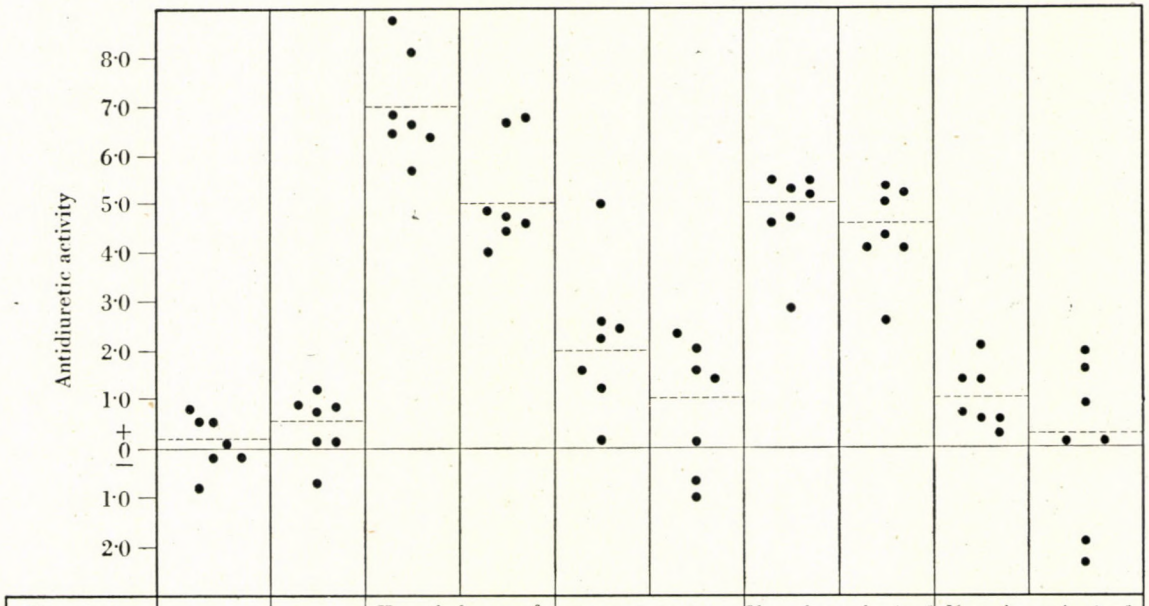
Group	Time after operation	Number of animals	Body weight g	Spontaneous water intake in 24 hours	
				ml	ml/100 g body weight
I. Intact	—	10	194.5 ± 6.0*	25.5 ± 1.7	13.4 ± 0.6
II. Stalk-lesions	6—14 days	10	208.5 ± 12.1	212.4 ± 14.6	101.1 ± 3.5
III. Stalk-lesions	more than 1 month	10	199.5 ± 6.3	179.1 ± 11.8	89.9 ± 5.1
		Probability:	I/II	$p \ll 0.001$	$p \ll 0.001$
			I/III	$p \ll 0.001$	$p \ll 0.001$
			II/III	$p > 0.05$	$p > 0.05$

* Standard error.

As to the hypothalamus, we shall only deal with changes in the supraoptic nucleus, as well as in the magnocellular portion of the paraventricular nucleus. No quantitative histological methods (counting of ganglionic cells, karyometry) were applied but it seemed as if the number of ganglionic cells was lower than normal and their size smaller than in the intact animals. The neurohypophysis showed marked atrophy, its size was significantly diminished. Beside the atrophy of the hypothalamus and neurohypophysis it was most conspicuous that the neurosecretory material had almost completely disappeared. In the magnocellular nuclei of the anterior hypothalamus, as well as in the neurohypophysis there were some occasional minute granules which took the stains applied. The lesioned hypophysial stalk showed no signs of regeneration; no new posterior pituitary-like structure developed.

Antidiuretic principle content of the hypothalamus and neurohypophysis

This was determined as already described [15, 20]. The animals were killed by decapitation and exsanguination. The hypothalamic (total hypothalamus) and neurohypophysial extracts prepared with physiological NaCl solution were injected intraperitoneally into intact and previously water-loaded rats, drawing conclusions as to the antidiuretic principle content from the intensity of the inhibition of diuresis. The results of this experiment are shown in *Fig.* 2. It can be seen that the neurohypophysis and hypothalamus of the intact rats contain considerable amounts of the antidiuretic principle. The neurohypophysis of the rats stalk-lesioned several weeks earlier shows almost no, or very slight antidiuretic activity. The same applies to the hypothalamus of the animals stalk-lesioned several weeks beforehand.



Treatment applied to recipient animals	Phys. NaCl	Frontal brain tissue	Hypothalamus of control rats		Hypothalamus of rats with stalk lesions		Neurohypophysis of control rats		Neurohypophysis of rats with stalk lesions	
			5-fold dilution	10-fold dilution	5-fold dilution	10-fold dilution	5-fold dilution	10-fold dilution	5-fold dilution	10-fold dilution
Mean	0·18±0·21*	0·50±0·22	7·08±0·39	5·22±0·42	2·30±0·35	0·97±0·53	4·90±0·35	4·33±0·36	0·91±0·31	0·22±0·64

*standard error

Fig. 2. Antidiuretic activity of hypothalamus and neurohypophysis of intact rats and rats with stalk-lesions more than 1 month earlier

Effect of cortisone, physiological saline and ethanol on the summation urine output of rats stalk-lesioned several weeks before

As previous investigations had indicated that in most stalk-lesioned animals oral tap water administration led to polyuria only for a time after operation, and after the lapse of 1 or 2 months diuresis returned to the normal level, it seemed interesting to examine the influence exerted by different substances on the water metabolism of rats stalk-lesioned several weeks beforehand. One group of such rats was given 5 per cent body weight of tap water by mouth and treatment for 2 or 3 days with daily doses of 10 mg/rat of cortisone (Adreson, Organon) subcutaneously. A second and a third group was given 5 per cent body weight of physiological NaCl solution or 5 per cent ethanol, instead of tap water. The rats were placed one by one into metabolic cages, urine output was measured every hour for 8 hours, and the urine excreted was evaluated as "summation urine output". The results are presented in *Table IV*. The data therein indicate that the summation urine output of the rats stalk-lesioned several weeks earlier did not differ significantly from that of the non-operated controls in the case of oral tap water treatment. On the other hand, treatment with cortisone and the oral administration of physiological NaCl solution resulted in significant polyuria, and the oral administration of ethanol caused moderate polyuria.

Table IV

Effect of cortisone, physiological NaCl solution and ethanol on the summation urine output of intact rats and rats with stalk-lesions more than 1 month earlier

Group	Fluid administered	Treatment	Number of animals	Body weight g	Summation urine output	Probability
I. Intact	tap water	—	10	185.0 ± 7.5*	6.34 ± 0.22	I/II $p<0.01$ I/III $p<0.001$
II. Intact	tap water	cortisone	10	167.0 ± 3.1	7.67 ± 0.27	I/IV $p<0.001$
III. Intact	phys. NaCl	—	10	208.5 ± 7.7	3.13 ± 0.36	V/VI $p<0.001$ V/VII $p<0.001$
IV. Intact	ethanol	—	10	180.0 ± 5.5	8.20 ± 0.22	V/VIII $p<0.01$ I/V $p>0.05$
V. Stalk-lesions	tap water	—	10	194.0 ± 7.7	6.56 ± 0.37	II/VI $p<0.01$ III/VII $p<<0.001$
VI. Stalk-lesions	tap water	cortisone	10	160.5 ± 4.9	9.64 ± 0.63	IV/VIII $p>0.05$
VII. Stalk-lesions	phys. NaCl	—	10	185.0 ± 4.3	11.68 ± 0.87	
VIII. Stalk-lesions	ethanol	—	10	180.0 ± 5.2	8.02 ± 0.29	

* Standard error.

Discussion

The results suggest that the disturbance of water metabolism which develops in conjunction with the pituitary stalk lesion undergoes a gradual alteration. Following operation namely (except for the interphase of temporary oliguria beginning on the third day and lasting 1 or 2 days) the animals responded with marked polyuria to the oral administration of tap water, whereas this polyuric reaction was no longer demonstrable 1 or 2 months after operation, when diuresis did not exceed or only slightly exceeded the average for the non-operated controls in the majority of the animals, so that one might feel tempted to claim that the diabetes insipidus-like condition had ceased. However, the normalization of water metabolism is only virtual, because spontaneous water intake continues to be increased, and the specific gravity and electrolyte concentration of the urine are lower than normal.

It was remarkable that the two methods of proven value for studying water metabolism (the diuretic reaction in response to the administration of water, and spontaneous water consumption) yielded divergent results in the rats stalk-lesioned several weeks earlier; the animals showing a normal diuretic response had namely polydipsia. Similar conclusions have been drawn from previous experiments concerned with the water metabolism of hypophysectomized rats [16]; these animals showed excessive antidiuresis in response to an oral water load, while spontaneous water intake did not decrease. It seems therefore that the changes in the diuretic reaction in response to an oral water load and those of spontaneous water ingestion are influenced by different mechanisms, and the examination of one parameter might show a pathological shift when the other yields normal results. This points to the fact that no reliable conclusions concerning water metabolism should be drawn on the basis of results obtained by one single method of assay.

The question arises, what factors take part in the production of the changes in water metabolism following stalk lesion. It is hardly questionable that the diabetes insipidus-like condition developing shortly after operation can be explained by a deficiency of antidiuretic hormone; it is unclear, however, why the characteristic polyuric response disappears 1 or 2 months later.

The first possibility is that several weeks after the stalk-lesion ADH production is resumed and this would be responsible for the apparent normalization of water metabolism. The basis for this hypothesis has been supplied by the investigations of STUTINSKY [33, 34], BILLENSTIEN and LEVEQUE [3], and MOLL [28, 29]. These authors studied serial sections of the hypothalamus and hypophysial stalk from animals hypophysectomized several weeks earlier and found that the broken stalk was capable of regeneration and a new, neurophypophysis-like structure developed in the stump. These morphological observations were supported by the results obtained by LLOYD et al. [24, 25],

as well as MIRSKY et al. [27], who found a re-appearance of ADH in the blood of animals after hypophysectomy performed several weeks earlier.

However, our investigations indicate definitely that no secretion of antidiuretic hormone can be demonstrated several weeks after stalk lesion and thus ADH can play no role in the cessation of the polyuric reaction. The rats stalk-lesioned several weeks earlier continued namely to excrete urine of low specific gravity and low electrolyte content, the antidiuretic activity of their hypothalamus was very low and in the neurohypophysis almost nil. The claim that the production of ADH would be resumed several weeks after destroying the stalk has not been supported by our morphological findings, either; we namely found marked atrophy, a disappearance of the neurosecretory material from the supraoptic and paraventricular nuclei, stalk, neurohypophysis system, and the stalk showed no sign of regeneration. Our previous experiments [22], too, yielded evidence ruling out the role in the process of ADH; we had found namely that acetylcholine, which liberates ADH, caused only a slight inhibition of diuresis in stalk-lesioned rats. Finally, we should mention the results of the ethanol experiments. It is known [8, 9, 10] that the administration of ethanol is followed by polyuria, and the increased diuresis is thought to be due to an inhibition of ADH production. The fact that following the administration of ethanol diuresis increased also in the animals stalk-lesioned several weeks beforehand does not prove the role of ADH, because ethanol may influence water metabolism in other ways, too. It therefore seems that, unlike after hypophysectomy, after stalk lesion it is just the tissues capable of regeneration that are destroyed and thus it is impossible that ADH production be resumed.

The cessation of the polyuric reaction may be brought into correlation first of all with the decrease of the glomerular filtration rate. It is known from the investigations of BERLINER and DAVIDSON [2] and other authors [14] that in diabetes insipidus the decrease of the GFR causes a diminution of polyuria. In previous experiments [17, 19] we, too, showed that in rats suffering from diabetes insipidus and dehydrated by water deprivation polyuria ceases completely, and may turn into oliguria if haemoconcentration increases and the circulating blood volume decreases to a considerable extent.

Our investigations have not clarified the mechanisms responsible for the decrease of GFR following stalk-lesion. From this point of view it is first of all a hypofunction of the adenohypophysis, of the ACTH-adrenocortical system that should be taken into consideration. There is namely a body of evidence [4, 30, 31] indicating that hypophysectomy and adrenalectomy result in a diminution of glomerular filtration, and after stalk lesion the pituitary-adrenal axis shows hypofunction [13]. We, too, have examined the adrenocortical function of rats several weeks after stalk lesion, by analysing the corticosterone content of adrenal venous blood; such animals were found to secrete less corticosteroid [19]. This view is further supported by our observation that

several weeks after stalk lesion excessive polyuria could be induced by cortisone administration or the diabetes insipidus made to cease by subjecting the animals to bilateral adrenalectomy [23].

However, the hypofunction of the pituitary-adrenocortical system does not explain completely the disappearance of the polyuric reaction. After stalk lesion adrenal activity is diminished a few days after operation already, yet the polyuric reaction remains demonstrable for weeks. Another argument against the exclusive role of the pituitary-adrenocortical system is the fact that in some of the animals with stalk lesion the diuretic response to the oral administration of tap water is still increased after the lapse of several weeks, yet in these animals, too, pituitary-adrenal function is diminished. Thus, other mechanisms (diminution of circulating blood volume? shifts in electrolyte metabolism?) should also be taken into consideration. Further investigations are required to decide the issue.

REFERENCES

1. Alexander, C. S.: Proc. Soc. exp. Biol. (N. Y.) **99,** 142 (1958).
2. Berliner, R. W., Davidson, D. G.: J. clin. Invest. **35,** 690 (1956).
3. Billenstien, D. C., Leveque, T. F.: Endocrinology **56,** 704 (1955).
4. Boss, W. R., Osborn, C. M., Renzi, A. A.: Endocrinology **51,** 66 (1952).
5. Brod, J., Sirota, J. H.: J. clin. Invest. **27,** 645 (1948).
6. Brunner, H., Kuschinsky, G., Peters, G.: Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak. **228,** 578 (1956).
7. Chester Jones, I.: The Neurohypophysis. H. Heller, ed. Proc. 8th Symp. Colston Res. Soc. Bristol. **8,** 253 (1957).
8. van Dyke, H. B., Ames, R. G.: Acta endocr. (Kbh.) **7,** 110 (1951).
9. Eggleton, M. G.: J. Physiol. (Lond.) **101,** 172 (1942).
10. Eggleton, M. G.: J. Physiol. (Lond.) **108,** 482 (1949).
11. Folin, O., Wu, H.: J. biol. Chem. **38,** 81 (1919).
12. Gál, Gy., Mihály, S., Farkas, L.: Kísérl. Orvostud. **5,** 63 (1953).
13. Harris, G. W.: Neural Control of the Pituitary Gland. Arnold, London 1955.
14. Kleeman, C. R., Maxwell, M. H., Rockney, R.: Proc. Soc. exp. Biol .(N. Y.) **96,** 189 (1957).
15. Kovács, G. S., Kovács, B. M., Kovács, K., Petri, G.: Acta med. Acad. Sci. hung. **12,** 201 (1958).
16. Kovács, K.: Die Rolle des Hypothalamus-Adenohypophysensystems im Wasserhaushalt. Studia Medica Szeged. In Press.
17. Kovács, K., Dávid, M. A., Horváth, I. W.: Endokrinologie **38,** 265 (1959).
18. Kovács, K., Dávid, M. A., Horváth, I. W.: Endokrinologie **38,** 278 (1959).
19. Kovács, K., Dávid, M., László, F.: Unpublished observations.
20. Kovács, K., Dávid, M., László, F.: In preparation.
21. Kovács, K., László, F. A., Dávid, M. A.: Endokrinologie **42,** 8 (1962).
22. Kovács, K., László, F. A., Dávid, M. A.: Endokrinologie **42,** 18 (1962).
23. László, F. A., Dávid, M. A., Kovács, K.: Endokrinologie. In Press.
24. Lloyd, C. W., Loewy, E., Pierog, S., Bradwick, K., Sostheim, R.: Proc. Soc. exp. Biol. (N. Y.) **85,** 333 (1954).
25. Lloyd, C. W., Pierog, S.: Endocrinology **56,** 718 (1955).
26. Lotspeich, W. D.: Endocrinology **44,** 314 (1949).
27. Mirsky, J. A., Stein, M., Paulisch, G.: Endocrinology **55,** 28 (1954).
28. Moll, J.: Z. Zellforsch. **46,** 686 (1957).
29. Moll, J.: J. Morphology **102,** 1 (1958).
30. Peters, G.: Nebennieren-Inkretion und Wasser-Elektrolythaushalt, Thieme, Leipzig 1960

31. PICKFORD, M., RITCHIE, A. E.: J. Physiol. (Lond.) **104,** 105 (1945).
32. SCHALES, O., SCHALES, S. S.: J. biol. Chem. **140,** 879 (1941).
33. STUTINSKY, F.: C. R. Soc. Biol. (Paris) **145,** 367 (1951).
34. STUTINSKY, F.: Z. Zellforsch. **39,** 276 (1953).

Dr. Kálmán KOVÁCS
Dr. Margit A. DÁVID
Dr. Ferenc A. LÁSZLÓ
Szeged, I. sz. Belklinika, Hungary

ÜBER DIE VERZÖGERUNG DER VOLUMWELLEN DER VENENPULSKURVE

Von

GY. BODROGI und A. KOVÁCS

HERZFÜRSORGESTELLE FÜR JUGENDLICHE (VORSTAND: DR. GY. BODROGI), BUDAPEST

(Eingegangen am 6. August 1962)

Die Venenpulskurve besteht größtenteils aus Volumwellen. Zur Fortpflanzung der Stromwelle ist eine gewisse Zeit notwendig. Über den vom Herzen distaler liegenden Venen erscheinen die Stromwellen verspätet, weshalb die einzelnen Punkte zur Zeitbestimmung ungeeignet sind. Am geringsten ist die Verzögerung des *v*-Gipfels, wenn die Abnahmestelle der Bulbus jugularis ist. Der aufsteigende Schenkel der *c*-Welle, die eine Druckwelle ist, erscheint unabhängig von der Entfernung vom Herzen, praktisch zum gleichen Zeitpunkt. Vom Bulbus jugularis kann die Venenpulskurve mit der photoelektrischen Methode nur mit Hilfe eines zwischengeschaltenen Fähnchens verfertigt werden. Mit dem von uns angewandten, auf Kapazitätsmessung beruhenden Verfahren kann meistens auch vom Bulbus ein zuverlässiges Mechanogramm bereitet und die Verspätung der Volumwellen auf ein Minimum vermindert werden.

Im allgemeinen werden die einzelnen Wellen der Venenpulskurve auch heute noch als Zeitsignalpunkte betrachtet, obwohl im letzten Jahrzehnt der zeitregistrierende Wert der Phlebogramme viel an Bedeutung verloren hat. Unserer Meinung nach sind zur Zeitmarkierung folgende Punkte geeignet: Unter Umständen der Beginn der *a*-Welle, manchmal der Beginn und der Gipfel der *c*-Welle. Diese sind nämlich Folgen der Druckveränderung und da zur Fortpflanzung der Druckveränderung eigentlich keine Zeit nötig ist, entstehen diese Punkte praktisch gleichzeitig mit den Ereignissen, die sie auslösen. Die übrigen Wellen, die Volumwellen sind, zeigen eine gewisse Verspätung. Das zeitliche Auftreten der entsprechenden Punkte der Volumwellen ist verschieden je nach dem, an welcher Stelle des Halses die Registrierung erfolgt.

Jahrelang wurde der Gipfel der *v*-Welle als Zeitpunkt der Mitralöffnung betrachtet. Dieser Zusammenhang diente zur Identifizierung des Öffnungstons, da man annahm, daß sämtliche, nach dem 2. Ton erfolgenden und gleichzeitig mit dem *v*-Gipfel zustande kommenden Tonerscheinungen Öffnungstöne sind, die am absteigenden Schenkel der *v*- bzw. *y*-Welle auftretenden Tonerscheinungen dagegen dem 3. Ton entsprechen. Diese Feststellung ist unrichtig, da die *v*-Welle als Volumwelle beträchtliche Verspätung aufweisen kann. LAGERLÖF und Mitarb. [4] stellten 1948 die Verwendbarkeit des *v*-Wellengipfels zur Tonidentifikation in Abrede. Es wurde nachgewiesen, daß die Verspätung zwischen *v*-Gipfel und Trikuspidalöffnung sogar 0,13″ ausmachen kann, so daß dieser erheblich verzögerte *v*-Gipfel eher den 3. Ton als den Öffnungston

anzeigt. Die Auffassung, daß jede mit dem v-Gipfel synchrone Tonerscheinung als Eröffnungston betrachtet werden soll, ist also grundsätzlich unrichtig.

ALTMANN und Mitarb. [2] bereiteten 1961 mit mehreren Methoden Phlebogramme synchron mit Vorhofkurven: An den mit verschiedenen Methoden registrierten Kurven zeigten sich zeitlich verschiedene Verzögerungen.

Wir befassen uns mit dieser Frage seit längerer Zeit. Zur Registrierung des Venenpulses verwenden wir ein »Dilatometer«, dessen Vorteil darin besteht,

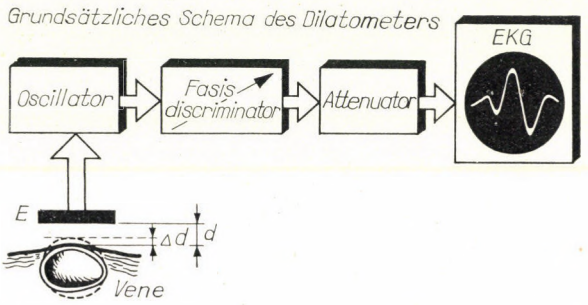


Abb. 1. Schematisches Bild des Apparates

daß der Rezeptor mit der Venenwand nicht in Berührung kommt. Das Instrument beruht auf dem bekannten Prinzip der Kapazitätsmessung und ist eigentlich ein Meßgerät zur Bestimmung kleiner Kapazitäten mit folgenden Bestandteilen: Elektrode mit etwa 0,5 mm² Oberfläche, welche in etwa 1—5 mm Entfernung von der Körperoberfläche liegt, ein Transistor-Oszillator mit 5 MHz-Frequenz, ein Frequenzdetektor sowie ein Attenuator. Der Apparat funktioniert nach dem in Abb. 1. dargestellten Schema. Eine oberflächliche Vene produziert der Herztätigkeit entsprechende Volumveränderungen, d. h. Pulsation. Durch die Pulsation wird die Entfernung d zwischen der über dem untersuchten Punkt befindlichen Elektrode E und der Körperoberfläche um Δd verändert. Die Elektrode und der Körper bilden einen Kondensator, dessen Kapazität von ihrem gegenseitigen Abstand abhängig ist. Die Abstandsänderung Δd verursacht eine Δc Kapazitätsveränderung, die den Schwingungskreis des mit der Elektrode verbundenen Oszillators verstimmt. Der Frequenzdetektor erzeugt eine der Verstimmung proportionale Spannung, die durch einen Attenuator unmittelbar an den Ekg-Apparat geschaltet werden kann. Es ist mathematisch nachweisbar, daß wenn das durch Pulsation verursachte Δd im Vergleich zu d genügend klein ist, das Gerät eine den Volumveränderungen proportionale Spannung ohne Berührung der untersuchten Körperoberfläche erzeugt; letztere würde durch mechanische Einwirkung die Meßwerte verfälschen.

Mehrere tausend mit diesem Apparat registrierte Venenpulskurven wurden überprüft. Wir fanden, daß der Gipfel der *v*-Welle meistens im erwarteten Zeitpunkt erscheint und zur Identifikation des Öffnungstons geeignet ist. Ziemlich häufig wurde jedoch eine hochgradige Verzögerung beobachtet.

Um zur Lösung der Frage näher zu kommen, wurden 200 Venenpulskurven von Kranken mit Mitralfehler analysiert; in 44% der Fälle war eine beträchtliche *v*-Verzögerung zu beobachten (Abb. 2 und 3). Interessanterweise war die

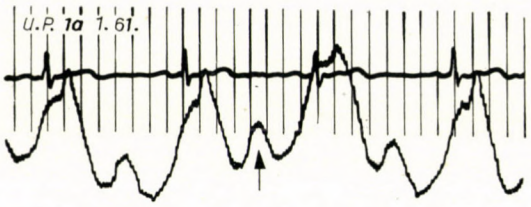


Abb. 2. Der Pfeil zeigt die Verzögerung der *v*-Welle

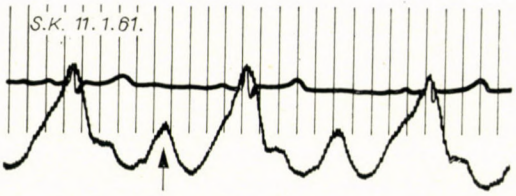


Abb. 3. Der Pfeil zeigt die Verzögerung der *v*-Welle

v-Verspätung in den Fällen am häufigsten, wenn die Registrierung nicht am Bulbus jugularis, sondern an einer ferner liegenden zervikalen Vene vorgenommen wurde. War die Abnahmestelle das unter dem Ohrläppchen liegende Gebiet, so war die *v*-Verzögerung beträchtlich. Da die Venenpulskurve anfangs an der am deutlichsten pulsierenden Stelle registriert und der Abstand vom Herzen nicht markiert wurde, konnte das ältere Material von diesem Standpunkt nicht bearbeitet werden. Damals wurde nämlich noch nicht damit gerechnet, daß zwischen den, an verschiedenen Halsregionen aufgenommenen Phlebogrammen, im Zeitpunkt des Erscheinens der einzelnen Wellen bedeutende Unterschiede bestehen können. Die genaue Markierung der Abnahmestellen erfolgte erst nachdem es erkannt wurde, daß die retrograde Druckschwellenfortpflanzung eine gewisse Zeit beansprucht.

Die Venenpulskurve wurde womöglich vom Bulbus jugularis aufgenommen, da hinsichtlich der äußeren Mechanographie diese Stelle recht herznahe liegt. Da sich der Bulbus tief zwischen den beiden Schenkeln des M. sternocleidomastoideus befindet, kann seine Bewegung mit der WEBERschen Methode nicht

registriert werden; dies ist nur mit einem zwischengeschalteten Fähnchen möglich. Dies bedeutet jedoch unserer Meinung nach einen Eingriff in die Venenvolumbewegungen und verursacht wahrscheinlich bedeutende Verzerrung. Mit Hilfe der Kapazitätsbestimmungsmethode waren wir jedoch häufig imstande, von dieser Stelle die Pulskurve abzuleiten, selbst wenn keine Venenbewegung augensichtlich war.

In 56 von 200 Fällen entstand der *v*-Gipfel im Vergleich zum 2. Herzton zum erwarteten Zeitpunkt. Die Registrierung erfolgte in sämtlichen Fällen

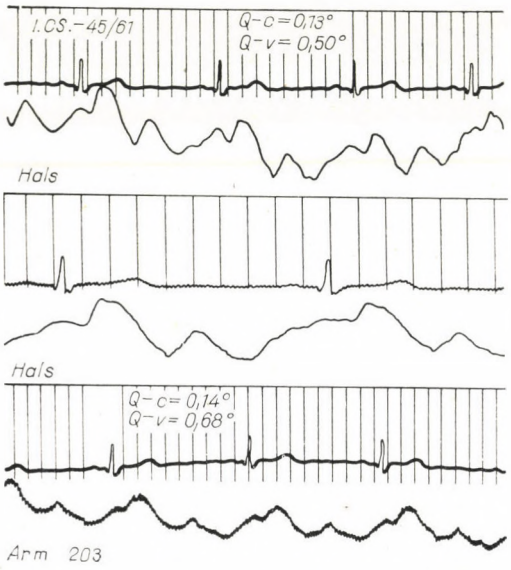


Abb. 4. Oben 2 zervikale Phlebogramme, unten Unterarmphlebogramm. Am Hals: *Q—c*-Strecke 0,13″, *Q—v*-Strecke 0,50″. Am Unterarm: *Q—c*-Strecke 0,14″, *Q—v*-Strecke 0,62″

am Bulbus jugularis. In den übrigen Fällen bestand eine Verzögerung der *v*-Welle. Die größte *v*-Verspätung war bei den Aufnahmen sichtbar, bei denen die Abnahmestelle unter dem Ohrläppchen, kranial vom Bulbus lag.

In 3 Fällen wurde an der linken Schulter Venenbewegung beobachtet. Die vom Bulbus jugularis und von dieser Vene verfertigten Aufnahmen ergaben folgende Werte:

I. Cs. 45/61: *Q—v*-Strecke an der zervikalen Vene 0,50″, an der Peripherie 0,62″. *Q—c*-Strecke an der zervikalen Vene 0,13″, an der Peripherie 0,14″ (Abb. 4).

I. G. 30/61: *Q—v*-Strecke über dem Bulbus jugularis 0,42″, an der Peripherie 0,48″ (Abb. 5).

In einem dritten Fall (B. P. 106/62) war am peripheren Phlebogramm ebenfalls wesentliche Verzögerung zu beobachten.

Diese 3 Fälle sprechen dafür, daß die *v*-Welle als Stromwelle sich langsam fortpflanzt und an der Peripherie mit meßbarer Verspätung erscheint. Die

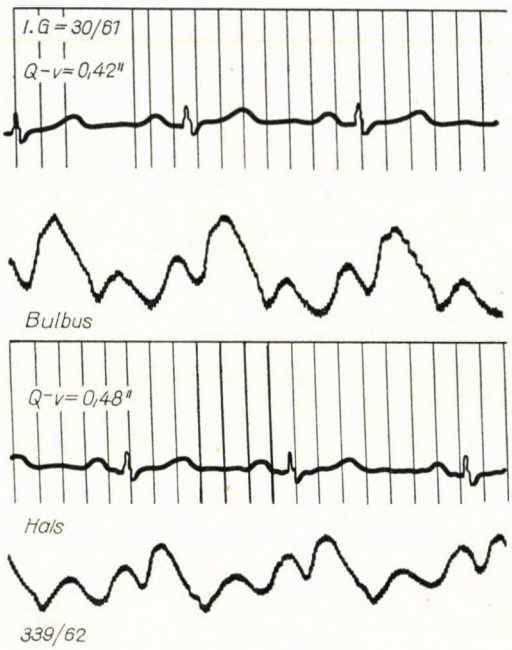


Abb. 5. Oben Bulbusphlebogramm, unten Phlebogramm des peripheren Halsteiles. *Q—v*-Strecke am Bulbus 0,42″, an der Peripherie 0,48″

c-Welle dagegen, die eine Druckwelle ist, erscheint gleichzeitig sowohl an der Peripherie als auch an der zervikalen Vene.

Besprechung

Am Phlebogramm wird außer der Druckveränderung in der Vene die Venenfüllung und -entleerung, d. h. ihre Volumveränderung registriert. Nur ein kleiner Teil der Wellen der Venenpulskurve kann als Druckwelle betrachtet werden, größtenteils sind es Volum- bzw. Stromwellen. Früher wurde jede Venenwelle als Druckveränderung betrachtet. Laut WENCKEBACH und Mitarb. [6] (1927) sind die Volumveränderungen für das Entstehen des Phlebogramms verantwortlich. GROEDEL [3] (1946) meint, daß außer den Druckveränderungen auch die Stromwellen von großer Bedeutung sind. LUISADA [5] (1954) analysierte die Venenpulskurve eingehend und nimmt an, daß sie teils Druck-, teils Volumveränderungen repräsentiert: seiner Meinung nach können beide auch innerhalb derselben Welle eine Rolle haben. ALTMANN [1] betonte (1958), daß die Venenpulskurve aus Volumwellen besteht. Auf Grund langjähriger Beobachtungen steht unsere Auffassung im Einklang mit der der Autoren, die sowohl Druck- als auch Volumveränderungen akzeptieren: Die aufsteigenden Schenkel der *a*- und *c*-Wellen sind Druckwellen, alle übrigen

sind Stromwellen. Die Fortpflanzungsgeschwindigkeit der *a*- und *c*-Wellen als Druckwellen ist recht groß, d. h. sie erscheinen an jeder Stelle der Vene praktisch gleichzeitig mit der sie auslösenden Herzfunktion. Da jedoch für die Fortpflanzung der Stromwellen — Ebbe- oder Flutwellen — eine gewisse Zeit notwendig ist, erscheinen diese Wellen an der Peripherie natürlich verspätet.

Der *v*-Gipfel steht mit der Mitralöffnung im Zusammenhang. Das Öffnen der Klappen ermöglicht die Strömung der Blutmenge aus dem Vorhof in die Kammer, dies verursacht im Venensystem und Vorhof Ebbe, die sich stufenweise entwickelt und vorerst proximal, später distal vom Herzen erscheint. Wegen der Inertie der Blutsäule und des Strömungswiderstandes benötigt die Ebbe Zeit zur Fortpflanzung.

Die Fortpflanzungsgeschwindigkeit der Ebbewelle, d. h. der Ebbe ist von der abstransportierten Blutmenge abhängig. Daraus läßt sich schließen, daß je schneller die Blutmenge aus dem Vorhof strömt, desto schneller entsteht die Ebbe und desto schneller erfolgt die retrograde Fortpflanzung der Ebbewelle. An einer näher zum Vorhof liegenden Stelle kann der Beginn der Ebbewelle natürlich früher registriert werden, als an distal liegenden Punkten. Damit kann erklärt werden, daß an Phlebogrammen der entfernt liegenden zervikalen Venen oder Schultervenen der Gipfel der *v*-Wellen signifikant später erscheint als z. B. an den über dem Bulbus aufgenommenen Kurven. Die retrograde Fortpflanzung der Ebbe- und Flutwellen kann natürlich gewissermaßen auch durch das Klappensystem beeinflußt werden. Dies ist individuell veränderlich, zweifellos sind aber meistens zwischen dem rechten Vorhof und dem Bulbus jugularis zahlreiche Klappensysteme vorzufinden. Das Gesagte liefert die Erklärung zur Beobachtung, daß die am Bulbus jugularis registrierte Venenpulskurve die *v*-Verzögerung in einigen Fällen erkennen läßt, in anderen dagegen nicht. Vermutlich ist die Fortpflanzungsgeschwindigkeit der Ebbewelle dort größer, wo weniger Klappen zwischengeschaltet sind, wodurch die Ebbe fast gleichzeitig mit dem — sie auslösenden — Öffnen der Trikuspidalklappe zustande kommt. Der Gipfel der *c*-Welle zeigt praktisch nie Verzögerung. Die 3 Schulterphlebogrammen zeigten praktisch keine Verspätung der *c*-Welle. Auch dies unterstützt die Annahme, daß der aufsteigende Schenkel der *c*-Welle eine Druckwelle darstellt.

Es ist somit offensichtlich, daß an der Venenpulskurve ausschließlich die Druckwellen zur Tonidentifikation angewendet werden können, die Volumwellen dagegen dazu ungeeignet sind. Je größer die Entfernung der Abnahmestelle vom Herzen, desto erheblicher ist die voraussichtliche *v*-Verzögerung.

LITERATUR

1. ALTMANN, R.: Kreislaufmessungen. I. Freiburger Colloquium, Dr. E. Banaschewski, München 1958.
2. ALTMANN, R., HENGST, W., HEINECKER, R., ZIPF, K. E.: Kreisl.-Forsch. **50**, 795 (1961).
3. GROEDEL, F. M.: The Venous Pulse and its Graphic Recording. Brooklyn Medical Press, New York 1946.
4. LAGERLÖF, H., WERKÖ, L.: Cardiologia (Basel) **13**, 241 (1948).
5. LUISADA, A. A.: The Heart Beat. Grune & Stratton, New York 1954.
6. WENCKEBACH, K. F., WINTERBERG, H.: Die unregelmäßige Herztätigkeit, Engelman, Leipzig 1927.

Dr. György BODROGI, Budapest V. Rosenberg hp. u. 27.
Albert KOVÁCS, Budapest VIII. Bérkocsis u. 23.

RESULTS OF LONG-TERM TREATMENT OF CHRONIC GLOMERULONEPHRITIS WITH CORTICOIDS

By

J. Brod, V. Fencl, Z. Hejl, J. Jirka and V. Prát

INSTITUTE FOR CARDIOVASCULAR RESEARCH (DIRECTOR: PROF. J. BROD)
PRAGUE, CZECHOSLOVAKIA

(Received October 23, 1962)

1. 33 patients with highly active chronic glomerulonephritis according to the criteria presented and 5 patients with low activity chronic glomerulonephritis were subjected to long-term therapy with cortisone or prednisone. 56 patients with highly active disease and 65 with a low activity, on the same regime but without corticoids, were used as controls. The control group did not differ from the treated group as regards age, the level of renal function at the start of observation, or the developmental trend in glomerular filtration. In the highly active group there was also no difference in the duration of the disease.

2. Under the influence of corticoids the clinical signs of activity of the disease decreased in both high and low activity groups.

3. In the high activity group, deterioration in glomerular filtration was arrested, and the trend reversed in significantly higher number than in the control group. In the low activity group the number of treated patients was too low for statistical evaluation.

4. The favourable influence of corticoid therapy usually lasted longer than the period of treatment, and in the majority of patients no relapse occurred in the follow-up period.

5. No serious complications were noted in the course of therapy.

Although the therapeutic value of corticoids in the nephrotic syndrome is well established, few studies seem to have been carried out so far on the possibility of influencing the course of chronic glomerulonephritis irrespective of the nephrotic syndrome. Farnsworth (1950) claimed to have achieved a favourable modification of the course of glomerulonephritis lasting from 6 weeks to 5 months in 3 children. Squire *et al.* (1957) remarked on the prolonged life expectancy of glomerulonephritis subjects whose nephrotic syndrome had been treated with corticoids. Armstrong and Kushner (1960) on the other hand warn against the use of corticoids, having noted the development of fatal uraemia in 1 adult patient whose NPN prior to treatment was 50 mg per 100 ml. The administration of cortisone to experimental animals with Masugi nephritis gave conflicting results; Enderlin *et al.* (1951) found marked morphological improvement, while Lippman *et al.* (1954) reported an enhancing effect on the gravity of the inflammatory signs. However, the experimental nephritis produced by nephrotoxic sera, though bearing many morphological and clinical similarities to human glomerulonephritis, also has some important differing features. In addition, it represents the early and not the later stage, when reparative scarring processes may compromise the function of the organ. Inhibition both of these processes and of the formation of antibodies, thought

to be important not only in the initiation of the disease, but also in the maintenance of activity (REJHOLEC *et al.*, 1952) is one of the prominent features of the effect of cortisone and allied corticoids. From this aspect, it would appear warranted to test the effect of these hormones in the later stages of glomerulonephritis.

Material and methods

The present study is based on a follow-up of patients with typical subacute or chronic glomerulonephritis, whose disease lasted at least 9 months (and usually more), and who were repeatedly observed in our Institute over at least 6 months (and usually more, the average duration of the follow-up being 2.4 years). The following investigations were carried out at each control follow-up, in addition to the usual history and physical examination: urine for quantitative protein analysis. Addis count, erythrocyte sedimentation rate, a 24-hour average glomerular filtration rate (endogenous creatinine clearance), and concentrating ability. This was estimated after withholding fluid for 36 hours, unless the subject attained an urine specific gravity exceeding 1028 in at least two successive 4-hour urine specimens before 36 hours. In both the treated and control group there was no difference in diet or other supplementary or symptomatic therapy. The period of bed-rest in both groups was comparable.

In the initial studies ACTH or cortisone were used, starting with an initial dose of 100 or 200 mg daily, followed by a sustaining dose of 50 or 100 mg daily; the total dose was 1050—9600 mg ACTH or 2000—3380 mg cortisone. Later, prednisone was mainly used in daily doses of 5—60 mg with a total dose of 0.54 to 6.97 g. The treatment was always carried out under penicillin screen, usually with streptomycin as well, and in the early group treatment was supplemented with 100 mg ACTH once a week.

Disease activity was assessed according to the criteria of BROD and BENEŠOVÁ (1957). These criteria take into account the daily rate of urinary excretion of protein, erythrocytes, leucocytes and casts, and the erythrocyte sedimentation rate, each factor being graded from 0 to 4 (Table I). The activity grade is expressed by the sum of points for all the five factors, so that the total can range from 0 to 20.

The progress of the disease was assessed from the changes in glomerular filtration rate. To make comparison easier, the latter data for a given individual were plotted against time, and a regression line was drawn. This was simple in most cases, but in some patients the data showed considerable scattering. In this instance we merely connected the points at the beginning and end of the observation period. To estimate the rate of change of glomerular filtration rate before the patient had come under our observation, we drew a line connecting the level of GFR of 120 ml/min (assumed to have been the normal glomerular filtration rate before the patient had fallen ill) at 0 time with the actual level of GFR found at the start of observation. The rate of change of glomerular filtration was expressed as per cent decrement (or increment) per annum. Changes exceeding ± 10 per cent were arbitrarily considered significant. The cor-

Table I

Point system for evaluation of activity of glomerulonephritis

Points	Proteinuria (gr/24 hours)	Addis count			Erythrocytes sedimentation
		Erythrocytes (millions)	Leucocytes (millions)	Casts (millions)	
0	0	0—1	0—2	0	1—12
1	0.1—1.0	2—10	3—10	0.1—0.5	13—20
2	1.1—3.0	11—30	11—20	0.6—1.0	21—30
3	3.1—5.0	31—50	21—50	1.1—2.0	31—40
4	>5.0	>50	>50	>2.0	>40

rectness of the impression thus gained concerning the progress of the disease was checked by the clinical assessment of the disease by an independent observer.

For purposes of analysis, patients with high and low activity disease were assessed separately, an activity grade of 10 having been taken as the arbitrary dividing line. Each of the two subgroups was compared with a group of untreated subjects with a corresponding degree of activity. The analysis was carried out both quantitatively and qualitatively. Quantitative analysis was carried out by Student's t-test (FISHER, 1946), qualitative analysis by the test of independence (II-test).

Results

The therapeutic studies were carried out in 38 patients with chronic glomerulonephritis. In 5, the disease was considered slightly active, whereas in 33 the activity grade was high. 121 patients with chronic glomerulonephritis were used as controls, 65 of which had low activity disease, and 56 had an activity grade of more than 10 points. In 4 patients the investigation was carried out in two separate periods, so that the total number of investigations amounted to 41 for the treated group and 122 for the control group.

There was no difference between the treated and untreated group in age, the initial level of glomerular filtration at the start of the observation period, or in the average developmental trend of glomerular filtration before the observation period had started (Table II). It can be seen from both Table II and

Table II

Clinical and functional data of patients with glomerulonephritis

	Chronic glomerulonephritis		
	Untreated	Treated	Statistical significance
		Low activity	
Age (years)	27.9	19.2	n
Duration of disease (years)	6.6	2.6	$p < 0.05$
Glomerular filtration rate before (ml/min.)	92.7	86.7	n
Aver. annual % change of GFR before	−13.7	−12.7	n
Aver. annual % change of GFR after	+ 3.3	+21.1	n
Change of activity	+ 0.05	− 5.0	$p < 0.01$
		High activity	
Age (years)	33.3	30.2	n
Duration of disease (years)	5.5	3.4	n
Glomerular filtration rate before (ml/min.)	66.3	71.2	n
Aver. annual % change of GFR before	−26.8	−29.4	n
Aver. annual % change of GFR after	−26.8	+13.7	$p < 0.001$
Change of activity	− 1.9	− 5.6	$p < 0.001$

Fig. 1 that the subdivision of the patients into two subgroups according to activity grade was in good agreement with the average developmental trend of the rate of glomerular filtration: —13.7 per cent and —12.7 per cent for the untreated or treated group with a low activity grade, and —26.8 per cent or —29.4 per cent for the subgroup with a high activity grade, respectively. The only significant difference between the subgroups was in the duration of

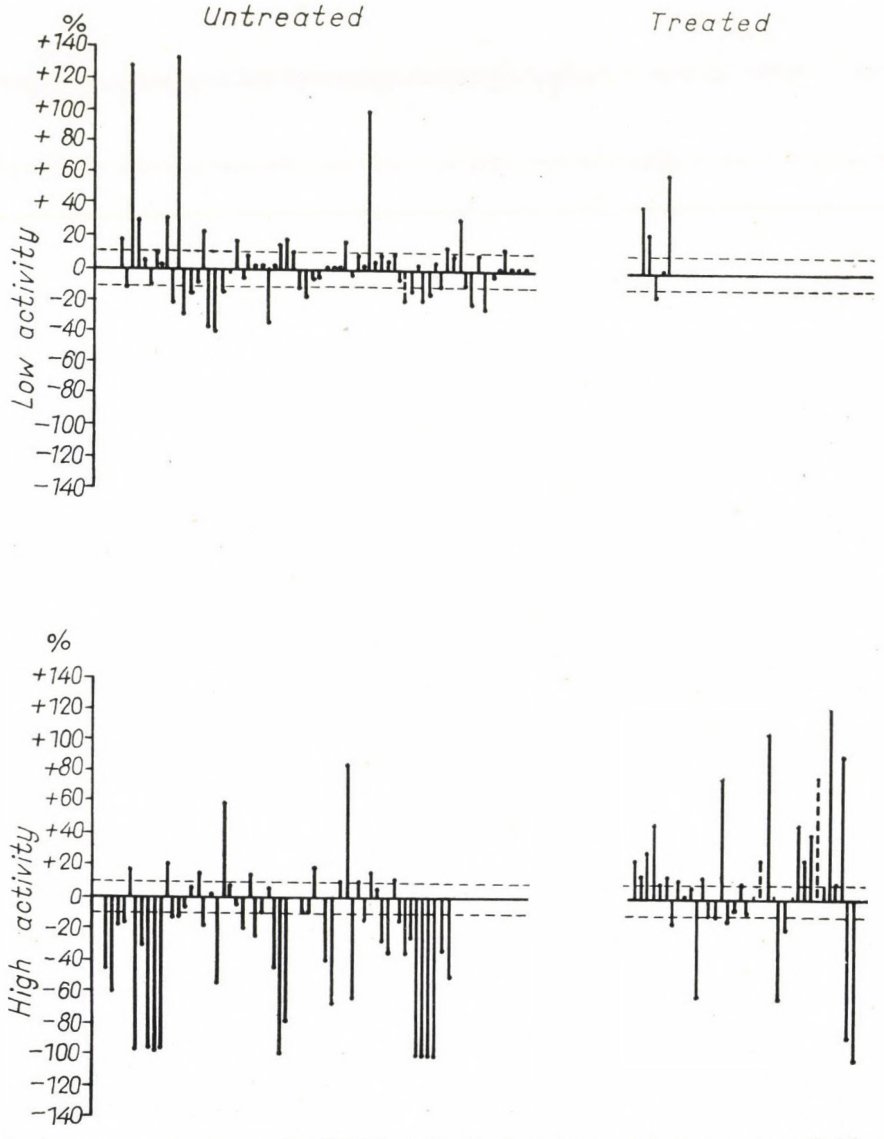


Fig. 1. Average yearly change in GFR in individual patients with glomerulonephritis with and without corticoid treatment. The horizontal dashed lines indicate the $\pm 10\%$ changes which were arbitrarily taken as the limit of significance

glomerulonephritis prior to the start of therapy, which was shorter in the treated group with a low activity grade than in the untreated one. However, in the more important subgroup with a high activity grade the difference in the duration of the disease between the treated and untreated subgroups was insignificant.

The influence of corticoids on the activity of the inflammatory process

There was, of course, considerable scatter in the data on the activity grade of the disease according to the clinical criteria referred to above. It was therefore necessary to judge the reproducibility of the results in patients who remained stationary over the course of several months or years. Of 48 such subjects, in whom GFR on repeated examination remained without change, in only 6 did individual activity ratings differ by more than 3 points from the average obtained from total assessment of activity grade in the entire group. A difference of ± 3 points from the average activity grade before treatment was therefore considered significant.

It can be seen from Table II that under the influence of corticoid treatment the activity grade decreased by 5.0 and 5.6 points for the low and high activity groups, respectively. The changes in both these subgroups differed significantly from the behaviour of the untreated subjects, where the average changes of activity of $+0.05$ and -1.9, respectively, were within the range of spontaneous variation of the activity grade.

The data in Table III demonstrate that in the group with low activity the rating decreased by more than 3 points in 16.9 per cent of the untreated, and in 80 per cent of the treated patients. In 13.9 per cent of the untreated subjects an increase in activity during the course of observation was found, whereas in the treated group an increase of activity was never observed. In the group with a high activity grade a similar drop in activity was noticed in 35.2 per cent of the untreated, against 71.5 per cent of the treated subjects. An increase of activity was noted in 12.95 per cent of the untreated and 2.8 per cent of the treated subjects.

In some of the subjects, in particular since we had started to apply higher doses, a temporary increase in proteinuria and Addis count was noticed. This, however, subsided when the corticoid dose was gradually lowered, and there were no later differences between these cases and the remainder of the clinical subgroup.

The influence of corticoids on glomerular filtration

It may be seen from Table II and Fig. 1 that in the high activity untreated group the decreasing tendency of glomerular filtration, found before observation, remained unchanged during observation (—26.8 per cent per year).

Table III

Effect of long-term corticoid therapy on glomerular filtration rate and disease activity

	Chronic glomerulonephritis														
	Untreated							Treated							
	No. of investigations	Change of glomerular filtration rate			Change of activity			No. of investigations	Change of glomerular filtration rate			Change of activity		
		Decrease	No change	Increase	Decrease	No change	Increase		Decrease	No change	Increase	Decrease	No change	Increase
		a	b	c	d	e	f		g	h	i	j	k	l
Low activity	66	17	35	14	11	45	9	5	1	1	3	4	1	0
					(only in 65 investig.)									
	%	25.7	53.1	21.2	16.9	69.2	13.9		20.	20.0	60.0	80.0	20.0	0
			74.3							80.0				
High activity	56	35	13	8	19	28	7	36	7	14	15	25	9	1
					(only in 54 investig.)							(only in 35 investig.)		
	%	62.5	23.2	14.3	35.2	51.85	12.95		19.4	38.9	41.7	71.5	25.7	2.8
			37.5							80.6				

Statistically significant differences in the rate of change of glomerular filtration rate between the untreated and treated patients with chronic glomerulonephritis of high activity grade: column

$$a : g \qquad p < 0.0005$$

$$c : i \qquad p < 0.01$$

$$(b + c) : (h + i) \qquad p < 0.0005$$

On the other hand, a marked difference may be noted in the group treated with corticoids, where the descending trend was replaced by an average increase o +13.7 per cent per annum. The difference between the treated and untreated group was highly significant ($p < 0.001$). From Table II it follows that while this reversal of trend occurred in 41.7 per cent of the treated group, it was seen in only 14.3 per cent of the untreated group. In active glomerulonephritis, treatment may be considered successful even if it does not lead to improved renal function, but merely results in stabilization. This latter criterion was fulfilled in 37.5 per cent of the untreated and 80.6 per cent of the treated subjects. The treated subgroup with a low activity grade contained only 5 subjects so that differences compared with the untreated group are difficult to evaluate. However, here too, the general trend of glomerular filtration in the treated group was +21 per cent per annum as compared with +3.3 per cent in the untreated group. In agreement with the general classification of the cases in this subgroup there was no difference in the favourable course of the disease between the treated and untreated groups i. e. glomerular filtration did not change.

Analysis revealed no difference in the therapeutic response between subjects whose disease was complicated by the nephrotic syndrome (Type B — Nephritis) and those without such a complication. Nor did we find any relationship between the result of treatment and the initial rate of change of glomerular filtration, although we usually did not administer corticoids to subjects whose initial glomerular filtration level was less than 30 ml/min.

No regular effect of the corticoid therapy on concentrating ability was observed.

Duration of the therapeutic effect

This could be assessed in 14 subjects who were under observation for a sufficient period after corticoid treatment had been terminated. The favourable effect lasted from 6 to 72 months, with an average of 22.5 months. In two subjects, new progression appeared after 17 and 48 months, respectively, while in the others no signs of such an unfavourable course were noted during their last follow-up visit.

Complications

In a majority of the subjects the usual acne, striae and cushingoid features appeared. Although rigid salt restriction was never applied (with the exception of cases with marked nephrotic syndrome) we never observed a significant rise in blood pressure. Neither were considerable changes in the mineral metabolism observed.

Discussion

Evaluation of the therapeutic effect in a chronic disease presents many difficulties, particularly in kidney disease where the pathological process has destroyed part of the parenchyma so that even full arrest of progression cannot bring about full functional restitution. Moreover, in a disease with an irregular natural history it is difficult to decide in an individual patient whether a certain change in the course of the disease is spontaneous or whether it should be regarded as a consequence of therapy. It was, therefore, necessary to use in the analysis statistical methods on a large material.

The primary function affected by the pathological process in glomerulonephritis is glomerular filtration. It is easy to estimate a given trend, if values found in a given patient in the course of a long observation period show little scatter about a straight line. Difficulties are encountered in those infrequent cases where values of glomerular filtration oscillate in both directions on repeated examination. That these oscillations were not due to the methods was evident from the good agreement of estimations carried out at short intervals. In subjects with an oscillating course of the disease, the developmental trend was judged from values at the beginning and end of the observation period. If the scatter of the data allowed the construction of two possible curves, we used the less favourable possibility in the treated group and the opposite in the untreated group. This procedure prejudiced the result against a favourable effect of treatment.

There might have been distortion of the results if the annual percentual change in glomerular filtration was assessed from different starting levels, as was certainly the case for the period after the start of observation. However, in the entire material (Table II) there was no significant difference in the levels of glomerular filtration at the start of observation between treated and untreated groups. Moreover, from the clinical point of view the percentual change in glomerular filtration rate within a given period of time is a better indicator of the significance of this factor than the tangent of the entire time curve. Thus, a decrement of glomerular filtration of 10 ml per annum gives the same slope in the ranges from 110 to 100 ml/min., 20 to 10 ml/min. and 15 to 5 ml/min., but the percentual decrease in the first instance is 8.5, in the second 50, and in the third 66.

The results of the present paper confirm the validity of the criteria for evaluation of the activity of the disease. The incidence of arrested disease was far greater in the low activity group and the rate of deterioration of glomerular filtration in this group was only half that in the high activity group.

The treated and untreated groups were comparable as regards age, duration and average severity of disease.

The results demonstrate that after a protracted course of corticoids, the clinical signs of disease activity decreased in the majority of cases and the

decreasing tendency of GFR was replaced by a rising trend. This result was highly significant in the high activity group. In the low activity group corticoid therapy was applied only to a small number of subjects, which explains why the results were statistically insignificant, although they were in good agreement with the above.

If precautions are taken, protracted corticoid therapy in adults does not lead to serious complications. The results of the present study suggest a favourable influence of corticoids on the intensity of the inflammatory process in the kidneys. They also demonstrate that it is possible in a high percentage of cases to arrest deterioration of glomerular filtration. We suggest this therapy to be indicated in all patients with a highly active disease. The small number of patients in the early stage of this therapeutic trial, when small corticoid doses were given, do not allow statistical analysis of the problem of dosage, nevertheless we would recommend a high initial dose, a long term course of therapy, and a high total dose. In the cases responding favourably, the change in trend of glomerular filtration becomes apparent within four weeks from the start of treatment, while changes in proteinuria and Addis count may appear before or after the glomerular filtration response has been noted. Treatment must be individualized.

We have refrained from using corticoids in renal failure, when most of the glomeruli have been replaced by scar tissue and theoretically no response could be expected. The increase in catabolism due to corticoids could under these conditions accelerate the development of uraemia. However, we do not possess sufficient data to substantiate this statement.

BIBLIOGRAPHY

ARMSTRONG, S. H. and KUSHNER, D. S.: Amer. J. Med. **29**, 377 (1957).
BROD, J. and BENEŠOVÁ, D.: Acta med. scand. **157**, 23 (1957).
ENDERLIN, M., ZOLLINGER, H. N., SPÜHLER, O. and WIFF, H.: Helv. med. Acta **18**, 525 (1951).
FARNSWORTH, E. B.: Proc. Soc. exp. Biol. (N. Y.) **74**, 54 (1950).
FISHER, R. A.: Statistical Methods for Research Workers. 10th ed., Stechert, New York (1946).
LIPPMAN, R. W., MARTI, H. N., JACOBS, E. E. and CAMPBELL, D. H.: Arch. Path. **57**, 405 (1954).
REJHOLIC, V., VANČURA, A., WAGNER, V. and MANDLÍKOVÁ, Z.: Sbor. lék. **54**, 195 (1952).
SQUIRE, J. R., BLAINEY, J. D. and HARDWICKE, J.: Brit. med. Bull. **13**, 43 (1957).

Dr. J. BROD, Praha-Krč, Budejovichá 800, ČSSR

ÜBER DIE UNTERSUCHUNG DER NIERENLYMPHE NACH URETERVERSCHLUSS

Von

M. PAPP

Technische Assistentin:
Marianne T. KOVÁCS

ABTEILUNG FÜR PATHOPHYSIOLOGIE (DIREKTOR: PROF. I. RUSZNYÁK),
FORSCHUNGSINSTITUT FÜR EXPERIMENTELLE MEDIZIN DER UNGARISCHEN AKADEMIE
DER WISSENSCHAFTEN

(Eingegangen am 10. Dezember 1962)

Lymphfluß und -zusammensetzung der innervierten bzw. denervierten Niere wurden vor und nach Ureterverschluß untersucht. Der Lymphfluß ergab identische Werte in Vorperioden der innervierten und der denervierten Niere, nach Ureterverschluß dagegen nahm der Lymphfluß der denervierten Niere signifikant zu ($p < 1\%$). Im Ausbleiben der Lymphflußsteigerung der innervierten Niere nach Ureterverschluß wird dem Lymphangiospasmus Bedeutung beigemessen. Im Falle von Ureterverschluß + Stauung der V. renalis aber nimmt der Lymphfluß von der innervierten Niere signifikant zu ($p < 0{,}1\%$). Der Lympheiweißgehalt nahm in allen Fällen nach Ureterverschluß sowohl in der innervierten ($p = 0{,}9\%$), als auch in der denervierten Niere ($p < 0{,}1\%$) signifikant ab. Der Lympheiweißgehalt der innervierten Niere war nach Stauung der V. renalis im Vergleich zu dem nach Ureterverschluß signifikant ($p < 1\%$) höher. Der *A/G*-Quotient in der Nierenlymphe war höher als im Blutplasma ($p < 1\%$). Die osmotische Konzentration der Lymphe sowohl der innervierten, wie der denervierten Niere war bei Ausscheidung von hypertonischem Harn bzw. nach Ureterverschluß konstant. Die osmotische Plasmakonzentration in der denervierten Gruppe war im Vergleich mit den anderen Versuchsgruppen am niedrigsten. In dieser Gruppe zeigte die Nierenlymphe im Vergleich zum Blutplasma Hyperosmolarität. Die osmotische Konzentration der Nierenlymphe wurde durch Stauung der V. renalis nicht beeinflußt. Der Zusammenhang zwischen Nierenfunktion und Nierenlymphzirkulation wird erörtert.

Nach Ureterverschluß spielen die Nierenlymphgefäße im Aufrechterhalten der Nierenfunktion eine wichtige Rolle [1, 2, 3, 4, 19]. Bei Hydronephrose erweitern sich die Nierenlymphgefäße, unter anderem infolge der erhöhten Lymphmenge. Nach Ureterverschluß gelangt aus dem Hohlsystem Harn in das Niereninterstitium. BABICS und RÉNYI-VÁMOS [1, 2] sind der Meinung, daß die vermehrte interstitielle Flüssigkeit durch das Nierenlymphgefäßsystem abtransportiert wird.

Die Nierenlymphe besteht aus Rinden- und Marklymphe. Das Gemisch der zwei Komponenten wird durch die Hilus- bzw. Kapsellymphkollektoren abtransportiert. Zwischen den zwei Lymphgefäßsystemen besteht eine anatomische und funktionelle Anastomose [3, 9]. Laut DRINKER [5] kann aus der Analyse der Lymphe auf die Zusammensetzung der interstitiellen Flüssigkeit gefolgert werden. Bei Absonderung hyperosmotischen Harns ist der osmotische Druck des Nierenrindeninterstitiums mit dem des Plasmas identisch, der des Markinterstitiums ist dagegen hypertonisch [6, 7, 8, 18). Der osmotische Druck der Nierenlymphe wird also durch die Zusammensetzung der isosmotischen Rinden- und hyperosmotischen Marklymphe bestimmt.

In unseren Experimenten untersuchten wir folgende Probleme:

1. Osmotische Konzentration der Nierenlymphe, des Blutplasmas und Harns bei hyperosmotischem Harn.

2. Veränderungen der Menge, der Zusammensetzung und des osmotischen Drucks der Nierenlymphe nach Ureterverschluß bei hyperosmotischem Harn.

Methodik

Die Versuche wurden an mit Pentobarbital narkotisierten (0,03 g/kg), hungernden, durchschnittlich 16 kg wiegenden Hunden beiderlei Geschlechts unternommen. Hydration: 70 ml/kg physiologisches Salz+5%ige Dextroselösung intravenös vor der Operation bzw. 70 ml/kg Wasser peroral 24 Stunden vor der Operation. 1%ige Lidocain-Tröpfelung der durch Medianlaparotomie freigelegten linken Niere. Kanülierung eines Hilus-, zuweilen Kapsellymphgefäßes, Harnsammlung aus dem linken Ureter mit Polyäthylenkanüle. Zwei Versuche wurden in Mannitdiurese durchgeführt. 0,05 ml/kg Heparinlösung diente zur Hemmung der Blutgerinnung und Heparinpulver zur Gerinnungshemmung der Blut- bzw. Lymphproben. Der Blutdruck wurde in der A. femoralis gemessen. In einer anderen Versuchsgruppe wurde der linke Ureter in Inactinnarkose aseptisch verschlossen. Nach 24 Stunden Lymphsammlung aus einem Lymphgefäß des linken Nierenhilus.

In 11 Fällen wurden die Experimente nach Denervierung der Niere vorgenommen, da anzunehmen war, daß die Nierenfreilegung — ähnlich wie in den Nierenblutgefäßen [10, 10a] — einen Spasmus der Lymphgefäße hervorruft [11].

Methodik der Nierendenervierung: Durchtrennung der zur Niere laufenden Nerven, Pinselung der A. renalis mit Phenollösung und abs. Alkohol nach Entfernung der Gefäßadventitia.

I. Durchführung der Experimente an innervierter (nicht denervierter) Niere:

1. 2—3stündige Sammlung der normalen Nierenlymphe und des Harns (Vorperiode).
2. Verschluß des Ureters.
3. 3—4stündige Lymphsammlung.
4. Verengung der linken V. renalis.
5. 30—60minutige Lymphsammlung.
6. Blutentnahme aus der A. femoralis zu Beginn und am Ende des Versuches.
7. Blutentnahme aus der linken V. renalis am Ende des Versuches.

In der nach 1tägigem Ureterverschluß untersuchten Gruppe dauerte die Lymphsammlungsperiode ebenfalls 3—4 Stunden. Darauffolgend Verengung der linken V. renalis, schließlich Lymphsammlung (30—60 Min.). Blutentnahme aus der A. femoralis, Blutgerinnungshemmung mit Heparinpulver.

II. Durchführung der Experimente an denervierter Niere:

Nach Nierendenervierung dieselbe Periode als bei Experimenten an innervierter Niere.

Chemisch analysiert wurde das Blutplasma der Arterie und Vene, außerdem der Harn vor dem Experiment bzw. der stauende Harn des verschlossenen Hohlsystems. Die osmotische Konzentration wurde durch Bestimmung der Gefrierpunktserniedrigung (Beckmann-Thermometer) gemessen. Eiweiß wurde mit dem Mikro-Kjeldahl-Verfahren mit Destillation bzw. Biuretverfahren [12] der Albumin-Globulin-Quotient mittels Papierelektrophorese bestimmt [12].

Die mathematisch-statistische Auswertung erfolgte mit Students »*t*«-Probe bzw. dem Vorzeichen-Test. Die Untersuchungen wurden mit Selbstkontrolle durchgeführt.

Ergebnisse

Tabellen I.—V. zeigen die Lymphwerte der innervierten bzw. denervierten Niere, außerdem die Blutplasma- und Harnwerte.

I. Lymphfluß aus der innervierten Niere :

a) Nach Ureterverschluß: In der mit physiologischer Salzlösung vorhydratierten Gruppe (Tab. I.) steigerte sich der Lymphfluß in 4 von 9 Experi-

Tabelle I

Lymphfluß aus der innervierten Niere, Eiweißgehalt der Nierenlymphe und des Blutplasmas vor und nach Ureterverschluß bzw. bei Stauung der V. renalis
(Vorbehandlung der Tiere mit physiol. Kochsalzlösung)

Nr.	Plasma			Nierenlymphe									Diurese in der Vorperiode
	A. femoralis		V. renalis	Normal			Ureterverschluß			Stauung der V. renalis			
	Gesamtprot. g%	A/G	Gesamtprot. g%	Fluß ml/min	Gesamtprot. g%	A/G	Fluß ml/min	Gesamtprot. g%	A/G	Fluß ml/min	Gesamtprot. g%	A/G	Fluß ml/min
1.	5,89	0,5	—	0,01	1,74	2,4	0,02	1,64	0,5	0,20	2,68	0,8	0,14
2.	7,65	0,7	7,20	0,20	1,66	1,5	0,15	1,22	1,5	0,54	3,81	0,8	0,48
3.	6,50	1,7	6,25	0,10	3,87	2,2	0,16	3,15	2,0	3,00	3,75	1,4	0,13
4.	6,12	1,3	5,95	0,01	2,40	1,7	0,01	—	—	0,09	3,53	1,2	0,07
5.	5,14	1,1	4,82	0,01	3,61	1,2	0,01	2,62	1,2	0,14	3,10	1,7	0,34
6.*	6,35	1,0	5,95	0,07	3,48	1,5	0,09	1,85	1,7	0,33	4,30	—	0,15
7.	4,51	0,7	4,18	0,06	1,64	0,7	0,02	—	0,7	—	—	—	—
8.	5,53	2,4	5,12	0,02	2,87	2,2	0,02	2,05	2,4	0,10	3,69	1,5	0,14
21.*	5,74	0,9	5,34	0,04	2,05	1,3	0,07	1,64	0,8	—	—	—	0,10
$\bar{x}$	5,94	1,14	5,60	0,06	2,59	1,64	0,06	2,02	1,35	0,63	3,55	1,23	0,19
s	0,89	0,59	0,94	0,06	0,89	0,55	0,06	0,66	0,68	1,06	0,53	0,37	0,14

* Kapsellymphe.

Tabelle II

Lymphfluß aus der innervierten Niere, Eiweißgehalt der Nierenlymphe und des Blutplasmas 24 Stunden nach Ureterverschluß
(Vorbehandlung der Tiere mit physiol. Kochsalzlösung)

Nr.	Plasma			Nierenlymphe					
	A. femoralis		V. renalis	Hydronephrose (24 Stunden)			Stauung der V. renalis		
	Gesamtprot. g%	A/G	Gesamtprot. g%	Fluß ml/min	Gesamtprot. g%	A/G	Fluß ml/min	Gesamtprot. g%	A/G
9	5,02	1,3	4,51	0,01	3,07	1,4	—	—	—
10	5,33	0,6	5,12	0,01	2,05	0,8	0,03	1,90	0,8
11	4,51	1,1	4,30	0,02	2,05	1,1	—	—	—
12	5,53	1,4	5,12	0,01	3,40	2,1	—	—	—
13	5,53	1,0	4,92	0,02	2,25	1,7	0,03	2,87	2,8
$\bar{x}$	5,18	1,1	4,79	0,01	2,56	1,4	0,03	2,38	1,8
s	0,42	0,3	0,37	0,005	0,63	0,5	—	—	—

menten. In 2 Fällen war keine Veränderung, in 3 jedoch eine Verminderung nachzuweisen. Die Veränderung war nicht signifikant. Nach peroraler Wasserzufuhr (Tab. III.) nahm der Lymphfluß in 6 von 7 Experimenten zu, in einem verminderte er sich. Die Zunahme war an der Grenze der Signifikanz ($5\% < < p < 10\%$). In Mannitdiurese war der Anstieg des Lymphflusses nach Ureterverschluß bedeutend (Tab. IV.). Die transportierte Lymphmenge der verschlossenen Niere war nach 1tägigem Ureterverschluß (Tab. II.) normal (Tab. I. und III.).

b) In der innervierten Gruppe mit Ureterverschluß + Stauung der V. renalis steigerte sich der Lymphabfluß signifikant ($p < 0{,}1\%$).

Tabelle III

Lymphfluß aus der innervierten Niere, Eiweißgehalt der Nierenlymphe und des Blutplasmas vor und nach Ureterverschluß bzw. bei Stauung der V. renalis
(Vorbehandlung der Tiere mit peroraler Wasserzufuhr)

Nr.	Plasma			Nierenlymphe									Diurese in der Vorperiode ml/min
	A. femoralis		V. renalis	Normal			Ureterverschluß			Stauung der V. renalis			
	Gesamtprot. g%	A/G	Gesamtprot. g%	Fluß ml/min	Gesamtprot. g%	A/G	Fluß ml/min	Gesamtprot. g%	A/G	Fluß ml/min	Gesamtprot. g%	A/G	
26	6,15	2,8	6,15	0,05	5,01	3,5	0,08	4,03	4,0	0,36	4,30	3,1	0,08
28	5,43	1,1	4,35	0,02	3,28	1,8	0,04	3,07	1,5	0,25	3,28	—	0,01
31	5,63	1,5	5,12	0,01	3,69	1,5	0,02	2,87	2,6	0,03	2,87	—	0,33
32	5,27	—	4,73	0,04	2,87	—	0,02	2,05	—	0,13	3,33	—	0,08
33	6,25	0,5	4,71	0,01	2,87	0,07	0,02	2,87	0,8	—	—	—	0,22
34	5,12	1,1	4,92	0,03	—	—	0,06	—	—	—	—	—	0,09
37	4,92	2,2	4,66	0,02	—	—	0,03	—	—	—	—	—	0,07
$\bar{x}$	5,54	1,53	4,95	0,03	3,54	1,87	0,04	2,98	2,23	0,19	3,45	—	0,13
s	0,50	0,83	0,58	0,02	0,84	1,18	0,02	0,71	1,40	0,14	0,61	—	0,11

Tabelle IV

Lymphfluß aus der innervierten Niere, Eiweißgehalt der Nierenlymphe bzw. des Blutplasmas vor und nach Ureterverschluß während Mannitdiurese

Nr.	Plasma			Nierenlymphe						Mannitdiurese Vorperiode
	A. femoralis		V. renalis	Mannitdiurese			Ureterverschluß			
	Gesamtprot. g%	A/G	Gesamtprot. g%	Fluß ml/min	Gesamtprot. g%	A/G	Fluß ml/min	Gesamtprot. g%	A/G	Fluß ml/min
17*	4,92	1,3	4,30	0,02	2,46	—	0,33	2,05	2,5	2,01
18	4,71	1,04	4,71	0,03	3,64	1,6	0,18	2,46	1,5	1,45

* Kapsellymphe.

Der Lymphfluß aus der denervierten Niere (Tab. V.) nahm infolge des Ureterverschlusses in 9 von 11 Experimenten signifikant zu ($p < 1\%$). Die Lymphflußwerte der innervierten und denervierten Niere waren in der Vorperiode identisch.

Der Gesamteiweißgehalt im Plasma der A. femoralis war signifikant höher ($p < 0{,}1\%$), als in der V. renalis (Tab. I.—V.). Unsere Ergebnisse stimmen mit den Literaturangaben überein [20, 21].

Der Eiweißgehalt in der Nierenlymphe war nach Ureterverschluß in allen Fällen signifikant niedriger, als in der Vorperiode: In der innervierten Gruppe nach Vorhydratierung mit physiologischer Salzlösung war $p < 0{,}9\%$ (Tab. I.), in der innervierten Gruppe mit peroraler Wasserzufuhr $p < 5\%$ (Tab. III.), in der denervierten Gruppe $p < 0{,}1\%$ (Tab. V.). Bei Ureterverschluß + Stauung der V. renalis steigerte sich der Eiweißgehalt der Nierenlymphe im Verhältnis zu den nach Ureterverschluß beobachteten Werten signifikant ($p < 1\%$). Die *A/G*-Werte in der Nierenlymphe waren signifikant ($p < 1\%$) höher, als im Plasma (Tab. I., III., V.). Die Minutendiurese war in der denervierten Gruppe signifikant höher ($p < 5\%$), als in der innervierten Gruppe (Tab. I., III., V.).

Tabelle V

Nierenfluß aus der denervierten Niere, Eiweißgehalt der Nierenlymphe und des Blutplasmas vor und nach Ureterverschluß bzw. bei Stauung der V. renalis
(Vorbehandlung der Tiere mit physiol. Kochsalzlösung)

Nr.	Plasma			Nierenlymphe									Diurese nach Denervation Vorperiode
	A. femoralis		V. renalis	Nach Denervation			Ureterverschluß			Stauung der V. renalis			
	Gesamtprot. g%	A/G	Gesamtprot. g%	Fluß ml/min	Gesamtprot. g%	A/G	Fluß ml/min	Gesamtprot. g%	A/G	Fluß ml/min	Gesamtprot. g%	A/G	Fluß ml/min
14	6,80	—	6,72	0,03	3,28	1,7	0,10	2,66	1,3	0,84	3,28	1,1	0,08
15	6,81	—	6,73	0,12	3,28	1,1	0,16	3,21	1,3	—	—	—	1,00
16	5,12	0,5	4,71	0,01	3,29	1,2	0,04	3,07	1,5	—	—	—	0,12
19	3,64	1,4	3,48	0,01	2,46	1,3	0,05	1,64	1,0	0,42	2,46	0,6	0,20
22*	5,43	0,5	5,33	0,02	3,07	0,9	0,03	2,77	0,7	—	—	—	0,10
38	4,55	2,2	4,10	0,01	3,47	—	0,06	2,84	2,3	0,25	3,24	—	0,04
39	4,82	2,2	4,71	0,04	—	2,6	0,04	—	2,8	0,06	3,28	—	0,38
40	5,84	1,8	6,15	0,04	2,87	1,2	0,11	2,70	1,0	—	—	—	0,16
42	5,20	2,0	—	0,04	3,28	2,3	0,04	2,87	2,5	—	—	—	0,05
43	5,13	—	5,94	0,02	3,28	—	0,03	2,97	—	—	—	—	0,04
44	5,12	—	—	0,05	3,07	—	0,16	2,87	—	—	—	—	0,90
$\bar{x}$	5,31	1,51	5,32	0,04	3,14	1,54	0,07	2,76	1,60	0,39	3,07		0,28
s	0,92	0,74	1,15	0,03	0,29	0,61	0,05	0,43	0,75	0,33	0,40		0,34

* Kapsellymphe.

Die osmotischen Konzentrationswerte der Lymphe, des Plasmas und Harns sind in Tab. VI., VIII. und IX. dargestellt. Tabelle VII. und VIII. zeigen, daß in der innervierten Gruppe der Mittelwert der osmotischen Konzentration im Plasma von jenem in der Lymphe nicht wesentlich abwich. Auf Wirkung des Ureterverschlusses + der Stauung der V. renalis (Tab. VI. und VIII.), sowie nach 1tägigem Ureterverschluß (Tab. VII.) veränderte sich die Osmolarität der Nierenlymphe nur unbedeutend. In der denervierten Gruppe bestanden bedeutende Unterschiede zwischen der osmotischen Konzentration des Blutplasmas und der Nierenlymphe in der Vorperiode ($p < 0{,}1\%$), sowie nach Ureterverschluß ($p < 3\%$) (Tab. IX.). Bedeutende Abweichungen ($p < 0{,}1\%$) waren auch zwischen den osmotischen Plasmakonzentrationswerten der beiden Gruppen festzustellen. Die osmotische Konzentration der Lymphe zeigte unter verschiedenen Versuchsbedingungen keine wesentlichen Unterschiede. Die osmotische Konzentration des Harns war in allen Experimenten höher, als die der Lymphe bzw. des Plasmas. Nach Ureterverschluß war die osmotische Konzentration des Harns ungefähr in der Hälfte der Fälle etwas höher bzw. niedriger als vor dem Verschluß und in allen Fällen höher als die osmotische Plasmakonzentration (Tab. VI., bzw. VIII., IX.).

Den normalen Lymphfluß [9, 13, 14], die Wirkung der Venenstauung auf den Lymphfluß und auf den Eiweißgehalt der Nierenlymphe betreffend waren unsere Ergebnisse mit den Literaturangaben übereinstimmend [2, 4, 5,

Tabelle VI

Osmotische Konzentration der Nierenlymphe, des Plasmas und Harns vor und nach Ureterverschluß
(Innervierte Tiere mit physiol. Kochsalzlösung-Vorbehandlung)

Nr.	Plasma mOsm/l		Nierenlymphe mOsm/l			Harn mOsm/l	
	A. femoralis	V. renalis	Normal	Ureter-verschluß	Stauung der V. renalis	Vor Ureter-verschluß	Nach Ureter-verschluß
1	312	301	326	321	321	1556	748
2	344	344	307	307	307	560	648
3	326	326	296	296	296	1473	1290
4	357	348	375	—	370	1424	1059
5	321	312	312	321	312	951	1134
6*	317	317	307	317	317	1033	1183
8	344	359	339	339	339	575	690
21*	352	352	335	326	—	785	—
$\bar{x}$	334,1	332,4	324,6	318,1	323,1	1044,6	965
s	17,2	21,2	25,2	13,7	24,6	400,1	—

* Kapsellymphe.

Tabelle VII

Osmotische Konzentration der Nierenlymphe und des Blutplasmas 24 Stunden nach Ureterverschluß
(Innervierte Tiere, Vorbehandlung mit physiol. Kochsalzlösung)

Nr.	Plasma mOsm/l		Nierenlymphe mOsm/l		Harn mOsm/l nach Ureterverschluß
	A. femoralis	V. renalis	Hydronephrose (24 Stunden)	Stauung der V. renalis	
9	392	403	326	—	554
10*	312	312	331	—	661
11	321	321	331	—	348
12	370	360	370	—	511
13	361	361	331	331	1330
$\bar{x}$	351,2	351,4	337,8	—	680,8
s	33,8	36,4	18,1	—	380,0

* Kapsellymphe.

Tabelle VIII

Osmotische Konzentration der Nierenlymphe, des Plasmas und Harns vor und nach Ureterverschluß
(Innervierte Tiere mit peroraler Wasserzufuhr)

Nr.	Plasma		Nierenlymphe			Harn, Vorperiode
	A. femoralis	V. renalis	Normal	Ureterverschluß	Stauung der V. renalis	
26	352	357	335	335	317	596
28	316	312	331	331	331	1050
31	331	331	339	344	331	914
32	335	331	339	348	335	661
33	340	340	320	340	350	730
37	336	298	330	—	—	1230
$\bar{x}$	335	328,2	332,3	339,6	332,8	863,5
s	11,8	20,8	7,2	6,8	11,8	245,8

9, 14, 15]. Dasselbe bezieht sich auch auf die osmotischen Konzentrationswerte des Plasmas bzw. der Lymphe [16, 17].

Daten über den Nierenlymphfluß nach Ureterverschluß bzw. nach Denervation, sowie über die Osmolarität der Nierenlymphe waren in der Literatur nicht zu finden.

Tabelle IX

Osmotische Konzentration der Nierenlymphe, des Plasmas und Harns vor und nach Ureterverschluß
(Denervierte Tiere, Vorbehandlung mit peroraler Wasserzufuhr)

Nr.	Plasma mOsm/l		Nierenlymphe mOsm/l		Harn mOsm/l Vorperiode
	A. femoralis	V. renalis	Nach Denervation	Ureterverschluß	
14	307	312	339	307	693
15	307	312	331	331	321
16	321	321	339	317	960
19	285	301	357	324	940
22*	315	301	331	331	860
38	314	302	—	330	569
39	321	302	346	346	634
40	312	314	330	308	—
41	294	—	330	—	500
42	330	—	320	336	671
43	319	302	330	335	817
44	308	—	314	—	—
x	311,1	307,4	333,4	326,5	687,5
s	12,3	7,4	11,8	12,5	194,7

* Kapsellymphe.

Besprechung

Folgende Ergebnisse sollen hervorgehoben werden:

1. Die Steigerung des Lymphflusses der innervierten Nieren war nach Ureterverschluß nicht signifikant, in den denervierten Nieren konnte dagegen eine signifikante Zunahme des Lymphflusses festgestellt werden. Dies spricht dafür, daß infolge der Nierenhilusfreilegung ein Lymphgefäßspasmus entstehen kann, der nach Ureterverschluß gegen die Steigerung des Lymphflusses wirkt. Nach Ureterverschluß wird ein Teil des in das Interstitium gelangenden Harns sicherlich durch das Lymphgefäßsystem abtransportiert: Die experimentelle Demonstration dieses Prozesses ist jedoch infolge des Lymphangiospasmus erschwert. Bei der Abweichung kann auch der geringeren Minutendiurese der innervierten Niere Bedeutung zukommen.

2. Nach Ureterverschluß wurde die Nierenlymphe trotz des hyperosmotischen Harns nicht hyperosmotisch.

3. Nach Ureterverschluß nahm in allen Experimenten der Eiweißgehalt in der Nierenlymphe ab.

Die Resultate sprechen dafür, daß der in das Niereninterstitium gelangende hyperosmotische Harn infolge Verdünnung isosmotisch wird und daß die interstitielle Eiweißkonzentration abnimmt. Auf das bedeutende Maß der Verdünnung weist die Tatsache hin, daß obwohl der Eiweißgehalt der Nierenlymphe nach Ureterverschluß + Stauung der V. renalis höher wird, als der nach Ureterverschluß gefundene, die normalen Eiweißkonzentrationswerte jedoch nicht überschreitet. Ein Teil der interstitiellen Flüssigkeit wird durch die Blutkapillaren, ein anderer (eiweißhaltiger) Teil aber durch das Lymphgefäßsystem abtransportiert.

Die osmotische Konzentration der Nierenlymphe ist auch unter verschiedenen Versuchsbedingungen ziemlich beständig und stimmt mit der des Blutplasmas überein. In der denervierten Versuchsgruppe dagegen ist die Osmolarität im Blutplasma niedriger als in der Nierenlymphe derselben und im Blutplasma der innervierten Gruppe. Die Ursache dieses Befundes ist unbekannt.

Neuerdings wird der Beobachtung eine bedeutende Rolle zugeschrieben, daß das Nierenmark im Vergleich zum Blutplasma hyperosmotisch ist [6, 7, 8, 18].

Die konstante und mit der des Blutplasmas übereinstimmende osmotische Konzentration der Nierenlymphe spricht dafür, daß keine bedeutende Lymphproduktion in der Medulla vor sich geht, oder aber wird die von hier stammende hyperosmotische Lymphe binnen kurzer Zeit isosmotisch. Eine andere Hypothese ist [22], daß die Lymphe aus der Medulla in Richtung der isosmotischen Rinde strömt: Damit kann die Isosmolarität der Nierenlymphe und des Blutplasmas leicht erklärt werden.

Schließlich soll noch die folgende Frage beantwortet werden: Welche Rolle kommt der Lymphzirkulation in der Nierenfunktion zu?

Dem kolloidosmotischen Druck der medullären Blutgefäßen und der Funktion der HENLEschen Schleifen ist im Aufrechterhalten der Hyperosmose des Markinterstitiums eine entscheidende Rolle beizumessen: Durch den kolloidosmotischen Druck der Kapillaren wird die während hypertonischer Harnbildung aus den Sammelröhren in das Interstitium gelangende Flüssigkeit abtransportiert. Die grundlegende Funktion der Nierenlymphgefäße ist, durch ständigen Transport des interstitiellen Eiweißes im Nierenmark den effektiven kolloidosmotischen Druck des Blutes zu sichern. Auf die Bedeutung des interstitiellen Eiweißtransportes weisen die Tatsachen hin, daß nach Verschluß der Nierenlymphgefäße einerseits die Diurese erhöht wird [23, 24], anderseits die osmotische Konzentration des Harns abnimmt [23].

* * *

Für die mathematisch-statistischen Analysen danken wir Herrn Béla Hajtmann auch an dieser Stelle.

LITERATUR

1. Babics, A., Rényi-Vámos, F.: Das Lymphgefäßsystem der Niere und seine Bedeutung in der Nierenpathologie und Chirurgie. Akademie Verlag, Budapest 1957.
2. Rényi-Vámos, F.: Das innere Lymphgefäßsystem der Organe. Akademie Verlag, Budapest 1960.
3. Földi, M., Romhányi, Gy.: Acta med. Acad. Sci. hung. **4,** 323 (1953).
4. Rusznyák, I., Földi, M., Szabó, Gy.: Physiologie und Pathologie des Lymphkreislaufes. Akademie Verlag, Budapest 1957.
5. Drinker, C. K., Yoffey, I. M.: Lymphatics, Lymph and Lymphoid Tissue. Harward. Univ. Press, Cambridge Mass. 1941.
6. Wirz, H., Hargitay, B., Kuhn, W.: Helv. physiol. pharmacol. Acta **9,** 196 (1951).
7. Ullrich, K. I., Drenckhahn, F. O., Jarausch, K. H.: Pflüg. Arch. ges. Physiol. **261,** 62 (1955).
8. Ullrich, K. I., Jarausch, K. H.: Pflüg. Arch. ges. Physiol. **262,** 537 (1956).
9. Sugermann, I., Friedmann, M., Barett, E., Addis, T.: Amer. J. Physiol. **138,** 108 (1942—43).
10. Gömöri, P., Munkácsi, I., Nagy, Z., Takács, L., Kállay, K.: Acta med. Acad. Sci. hung. **18,** 119 (1962).

10a. Gömöri, P., Nagy, Z., Jakab, J., Vajda, V.: Acta physiol. Acad. Sci. hung. **19,** 80 (1961).

11. Rusznyák, I., Földi, M., Szabó, Gy.: Acta Med. Scand. **137,** 37 (1950).
12. Bálint, P., Hegedüs, A.: Klinische Laboratoriumsuntersuchungen, Berlin 1962.
13. Szabó, Gy., Papp, M., Magyar, Zs.: Experientia (Basel) **18,** 128 (1962).
14. Le Brie, S. J., Mayerson, H. S.: Amer. J. Physiol. **198,** 1037 (1960).
15. Swann, H. G., Ormsby, A. A., Delashaw, I. B., Tharp, W. W.: Proc. Soc. exp. Biol. (N. Y.) **97,** 517 (1958).
16. Bottazzi, F.: Ergebn. Physiol. **7,** 1961 (1908).
17. Nakamura, A.: Lymphatologia (Kyoto) **1,** 67 (1952).
18. Lamdin, E.: Arch. intern. Med. **103,** 644 (1959).
19. Babics, A., Rényi-Vámos, F.: A vesepusztulás elmélete és klinikuma. Akadémiai Kiadó, Budapest 1952.
20. Shida, K.: zit. 21.
21. Harms, W. I., Blitch, P. G., Johnson, A. C., Findlay, T.: Metabolism **11,** 542 (1962).
22. Бочаров, В. Я-: Сб. трудов кафедра анатомии Ленингр. Санит. Гигиен. Мед: Инст. том. **35,** 164. 1957.
23. Kaiserling, H., Soostmeyer, Th.: Wien. klin. Wschr. **52,** 1113 (1939).
24. Földi, M., Romhányi, Gy.: Acta med. Acad. Sci. hung. **4,** 323 (1953).

Dr. Miklós (N.) Papp, Budapest VIII. Korányi S. u. 2. I. sz. Belklinika, Ungarn

EXPERIMENTELLE UNTERSUCHUNGEN ÜBER DIE VERÄNDERUNGEN DES EXTRAZELLULÄREN RAUMES BEIM STRAHLENSYNDROM

Von

Z. Zsebők und Gy. Petrányi jr.

MEDIZINISCH-RADIOLOGISCHE FORSCHUNGSGRUPPE
DER UNGARISCHEN AKADEMIE DER WISSENSCHAFTEN

(Eingegangen am 4. Januar 1962)

Bei Ratten wurden die mit dem durch 1500 r ausgelösten gastrointestinalen Strahlensyndrom parallel auftretenden Veränderungen des extrazellulären Raumes analysiert.

Während der ersten 24 Stunden nach der Strahlenexposition ist der extrazelluläre Raum recht labil und weist negative und positive Schwankungen auf. Einmalige Bestimmung des extrazellulären Raumes ergibt in dieser Zeitperiode kein reales Bild. Von der 24. Stunde an entstehen gastrointestinale Symptome und der extrazelluläre Raum vermindert sich zuerst sukzessiv, von der 65. Stunde bis zum Tode ist jedoch eine starke Senkung der extrazellulären Werte zu verzeichnen. In der gesamten Beobachtungszeit verminderte sich der extrazelluläre Raum um 54—58%.

Die Ergebnisse werden anhand der neueren Literaturangaben über das gastrointestinale Strahlensyndrom bewertet.

Die Untersuchungen von Schade und Menschel (1923) [24] haben gezeigt, daß nach Röntgenganzkörperbestrahlung im von Kapillarwand und Zellmembran begrenzten sog. »Dreikammersystem« des Organismus verschiedene Veränderungen zustandekommen, die teils durch die veränderte Permeabilität der Kapillarwand, teils durch eine Funktionsveränderung der osmotischen Zellmembran erklärt werden können. Auf Grund der irreversiblen morphologischen Veränderungen der Endothelzellen konnten Lazarew und Lazarewa [14] die Funktionsveränderungen der Kapillarwand nach Bestrahlung mit 600 r nachweisen. Im interstitiellen Raum kann lediglich die Permeabilitätsveränderung der Kapillarwand derartige Verschiebungen verursachen, worauf schwere Störungen im Salz- und Wasserhaushalt des ganzen Organismus erfolgen (Kerpel-Fronius [12]). Da jedoch nicht nur mit der direkten, sondern auch mit einer allgemeinen, aspezifischen Schockwirkung der Bestrahlung gerechnet werden muß, soll bei der Prüfung der Permeabilitätsveränderungen der die einzelnen Systeme trennenden Membranen, auch diese Tatsache berücksichtigt werden. Von den zahlreichen Forschern, die sich mit der Klärung der Einzelheiten des komplexen Schockmechanismus befaßten, erwähnen wir Cannon [4], Moon [17], Rusznyák, Karádi, Hámori [22, 23], Gömöri [9].

Da die Literaturangaben einander gewissermaßen widersprechen, war das Ziel vorliegender Arbeit, die sich zum Strahlensyndrom der Ratten gesellenden Veränderungen des extrazellulären Raumes mit genauem und zuver-

läßigem Verfahren zu bestimmen. Vor der Beschreibung der angewandten Methodik sollen die neueren Gesichtspunkte über den extrazellulären Raum und die diesbezüglichen Untersuchungen kurz behandelt werden.

Eine ideale Substanz, die in den intrazellulären Raum überhaupt nicht eindringt, steht zur Bestimmung der extrazellulären Flüssigkeit nicht zur Verfügung, woraus folgt, daß keine der zur Bestimmung des extrazellulären Raumes dienenden Methoden absolut exakte Ergebnisse liefert. Bei Anwendung von Inulin oder Mannit können jedoch ausreichend genaue Resultate gewonnen werden [7, 13, 19]. Die mit anderen Substanzen, wie z. B. Thiocyanat, Na- und Cl-Isotopen bestimmten Räume können nur als Na-, Cl- usw. Verteilungsräume bezeichnet werden. Ein unbedeutender Fehler der Inulinmethode ist die langsame Penetration der Substanz in den extrazellulären Raum der Elastin- und Kollagenelemente.

Laut COTTLOVE (zit. 16) verläuft die Inulinverteilung im extrazellulären Raum in zwei gesonderten, exponentiellen Diffusionsphasen. Wie bereits erwähnt, kann der extrazelluläre Raum als Dreikammersystem betrachtet werden, das aus einem aktiven und einem inaktiven Raum besteht. Der aktive extrazelluläre Raum besteht wieder aus zwei — leicht und schwer diffusiblen — Teilen, letzterer besteht aus dem Bindegewebe-, Knochen- und aus dem kleinen zellulären Inulinraum. Der inaktive bzw. transzelluläre Raum umfaßt die nicht zum Inulin-Verteilungsraum gehörenden Flüssigkeiten: Liquor, Galle, der in den Nieren befindliche Harn, die Gelenksflüssigkeit [15, 16].

Da sich das Inulin im aktiven extrazellulären Raum verhältnismäßig schnell und gleichmäßig verteilt, ist es zur Bestimmung des extrazellulären Raumes der kleinen Säugetiere außerordentlich geeignet [3, 7, 13]. Das Infusionsverfahren mit Harnsammlung, oder Nephrektomie mit einer Injektion verbunden sind zur genauen Bestimmung des Verteilungsraumes geeignet. Das erstere Verfahren ist aus der Literatur wohlbekannt [2, 13], das zweite beruht auf folgendem Prinzip: Da die Elimination bzw. intrazelluläre Diffusion des in einer Injektion verabreichten Inulins bei nephrektomierten Tieren viel langsamer und gleichmäßiger vor sich geht, kann die Verteilung exponentiell charakterisiert werden [19]. In bester Annäherung erhält man den Verteilungsraum, wenn die Inulinkonzentration im Serum zu verschiedenen Zeitpunkten nach der Inulingabe bestimmt wird. Werden die erhaltenen Werte oder die umgerechneten extrazellulären Räume im semilogarithmischen Maßstab dargestellt, so erhält man eine steil verlaufende Gerade, die auf den Zeitpunkt »0« extrapoliert wird. Nach den Untersuchungsergebnissen der letzten Jahre wurde bei Ratten für die extrapolierten extrazellulären Räume 16—15,3% gefunden, die maximale Schwankung beträgt 1,5—3% [19, 29].

Infolge der exakten Ergebnisse und der einfachen Ausführung fanden wir diese Methode — mit geringer Modifikation — für die Registrierung der Veränderungen des extrazellulären Raumes von bestrahlten Ratten geeignet.

Methodik

In den Vorversuchen wurde intakten Ratten nach Unterbindung des Nierenhilus eine große Inulindosis [100 mg/kg] in einmaliger Injektion verabfolgt [28], sodann die Inulinkonzentration im Serum zu verschiedenen Zeitpunkten bestimmt. Die durchschnittlichen Konzentrationswerte sowie die auf den extrazellulären Raum umgerechneten Werte wurden im semilogarithmischen Maßstab dargestellt, und auf den Zeitpunkt »0« extrapoliert. Auf diese Weise erhielten wir extrazelluläre Raum-Werte von 16,15% (Abb. 1). In sämtlichen, parallel

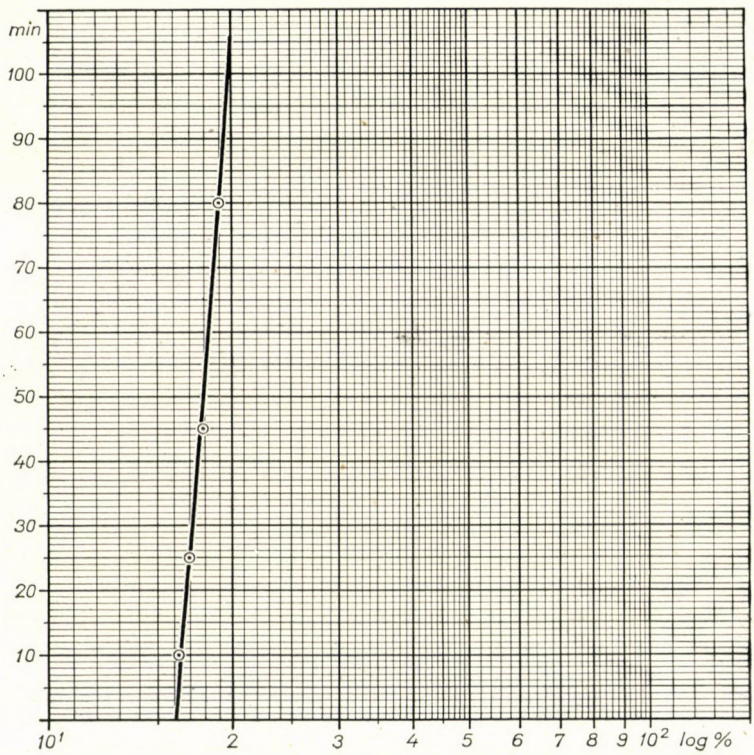


Abb. 1. Semilogarithmische Inulinwerte im Serum in verschiedenen Zeitpunkten (Einmalige Inulininjektion, Ratten mit Nierenhilusverschluß)

durchgeführten und wiederholten Untersuchungen ergab sich dieselbe exponentielle Verteilung. Da das Inulin sich im schnell diffundierbaren Teil des extrazellulären Raumes bei nephrektomierten Ratten binnen 20—30 Minuten verteilt [28], wurde die Inulinkonzentration des Serums und damit der extrazelluläre Raum in einer weiteren Versuchsserie nur in der 30. Minute bestimmt. Der gewonnene Durchschnittswert (16,4 $\pm$ 1,36%) war mit dem 30-Minutenwert der beschriebenen exponentiellen Verteilungsfunktion identisch. Infolge der geringen Streuung der Versuchsergebnisse, sowie der minimalen Abweichung zwischen dem auf »0« extrapolierten und dem 30-Minutenwert (0,25%) fanden wir für die Bestimmung des relativen extrazellulären Raumes die einmalige Blutentnahme nach 30 Minuten, mit doppelter Kontrolle von ausreichender Genauigkeit.

Die Versuche und die Vorversuche wurden an 121 Wistar-Ratten (1—2-monatige Tiere von 120—180 g Gewicht) beiden Geschlechts durchgeführt.

Während der Experimente erhielten die Tiere Futter bestimmter Menge und Zusammensetzung. Wasser erhielten sie ad libitum. Eine Tiergruppe erhielt keine Flüssigkeit, einer anderen Gruppe wurde 1 Stunde nach der Bestrahlung peroraler Wasserstoß (5% des Körpergewichtes) mit destilliertem Wasser verabfolgt.

Die Ganzkörperbestrahlung erfolgte mit einem STABILIPAN-Apparat (Siemens) und einer Dosisleistung von 35 r/min. Die Strahlendosis betrug 1500 r, HWL war 1,0 mm Cu. Während der Bestrahlung wurden die Tiere in aus Plexiglas verfertigten, mit Fächern isolierten Käfigen untergebracht. Die unter identischen experimentellen Bedingungen gehaltenen Kontrolltiere wurden — ohne daß sie der Strahlenwirkung ausgesetzt gewesen wären — eine gleiche Zeit lang in die Bestrahlungsgefäße gelegt.

Die extrazellulären Raumbestimmungen wurden 1, 6, 12, 24, 48 und 72 Stunden nach der Strahlenexposition vorgenommen, das Inulin wurde in diesen Zeitpunkten injiziert, die Nierenhilus-Abbindung erfolgte 30 Minuten vorher, die Exsanguination 30 Minuten nachher.

Nierenhilusabbindung: Äthernarkose, retroperitoneales Eindringen aus dorsalem Schnitt, Abtrennung der Nierengebilde; die Nebennieren blieben unberührt und der Nierenhilus wurde mit einem dünnen Faden abgebunden. Die Inzision wurde mit 1—2 Nähten, die Haut mit Klammern verschlossen. Da die extrazellulären Raumbestimmungen bei einer größeren Anzahl von Tieren zugleich durchgeführt wurden, erfolgte bei jedem Tier zuerst das Abbinden des Nierenhilus.

Inulin-Verabfolgung: Nach der Methodik von WHITE und ROLF [28] wurde den Tieren 30 Minuten nach dem Eingriff 1 ml 1%ige Inulin-Lösung (10 mg) in die Schwanzvene injiziert. Die Injizierung erfolgte in milder Äthernarkose, wodurch die gesamte Inulin-Menge restlos und sicher in den Kreislauf gelang. Versuche, bei denen die Injizierung paravenös geschah, oder geringere Blutungen entstanden, wurden nicht bewertet.

Exsanguination: Die Blutentnahme durch Herzpunktion wurde mit einem eigens zu diesem Zweck konstruierten, geschlossenen Saugsystem durchgeführt. Die Glas- und Gummibestandteile des Apparats wurden vorangehend von den anhaftenden Spuren von Inulin sorgfältig gereinigt. Das entnommene Blut wurde heparinisiert und unmittelbar zentrifugiert.

Inulin-Bestimmung: 1 ml Plasma wird nach SOMOGYI alkalisch enteiweißt (Verdünnung 1 : 10) [2]. 2 ml der eiweißfreien Verdünnung werden mit 0,5 ml 16%iger 4 n NaOH-Lösung versetzt und das Gemisch zur Zerstörung des Zuckers in ein kochendes Wasserbad gestellt, danach mit Leitungswasser abgekühlt. Man versetzt mit 5 ml Diphenylaminreagens und stellt die Reagenzgläser abermals für 30 Minuten in ein kochendes Wasserbad [19]. Nach Wasserkühlung wird binnen 20 Minuten mit dem Uvifot-Photometer, bei Filterstellung MG (dies entspricht der Wellenlänge 625 mμ) photometriert. Die Berech-

nungen wurden mit Standardlösungen mathematisch und graphisch parallel durchgeführt. Doppelte Kontrolle der erhaltenen Werte: Einerseits wurden aus jeder Blutprobe Parallelbestimmungen durchgeführt (im Fall von Inkongruenz wurde das Resultat nicht bewertet) anderseits wurde nach der bereits geschilderten Methode die exponentielle Kurve der Versuchsserien konstruiert und die 30-Minutenwerte mit den entsprechenden Punkten dieser Kurve verglichen. Es soll noch erwähnt werden, daß das zur Lösung des Inulins dienende Flüssigkeitsvolumen (1 ml) von dem gemessenen extrazellulären Flüssigkeitswert subtrahiert wurde. Zur graphischen Bewertung wurden die Extinktionswerte der Inulin-Verdünnungsserie in einer Gleichung zweiten Grades (mit der entsprechenden Fehlerkorrektion) ausgedrückt.

An der graphischen Kurvendarstellung der Gleichung sind auf Grund der Extinktionswerte die untersuchten extrazellulären Räume unmittelbar in ml abzulesen.

Besprechung

Die aktiven, schnell diffundierbaren extrazellulären Raumveränderungen wurden bei mit 1500 r bestrahlten Ratten zu verschiedenen Zeitpunkten bestimmt. Die Resultate sind in Tab. I. zusammengefaßt: 1 Stunde nach Strahlenexposition ist die Steigerung des extrazellulären Raumes nicht signifikant ($+$ 1,9%, $p < 0{,}20$), 12 Stunden lang erfolgt eine sukzessive Abnahme, in der 24. Stunde sind wieder ansteigende Werte zu verzeichnen (12 Stundenwert: 13,7%, 24 Stundenwert: 19,1%). Da nicht sämtliche erhaltenen Werte signifikant sind und z. B. die Standarddeviation des 6 Stundenwertes verhältnismäßig hoch ist ($\pm$ 2,70), kann man die Veränderungen des extrazellulären Raumes in den ersten 24 Stunden nur schwer charakterisieren. Es ist allerdings festzustellen, daß der extrazelluläre Raum in dieser Zeitperiode recht labil ist und seine Größe die Streuungsgrenzen übertreffende Schwankungen aufweist. Diese fast charakteristische Labilität findet ihre Erklärung in den am ersten Tag oft antagonistisch wirkenden Prozessen und Symptomen, die den Wasserhaushalt und seine Regulation beeinflussen, wie z. B. gesteigerte Kapillarpermeabilität [1, 25, 27, 29], Polyurie und Polydipsie [1, 10, 11, 18], Anstieg der Wasseraufnahmefähigkeit von strahlenempfindlichen Geweben und Dehydration anderer Gewebe [1, 5], gesteigerte Magensekretion in der 8—12. Stunde [1]. Die Richtung der Verschiebung der Wasserräume kann lediglich auf Grund der extrazellulären Raum-Veränderungen nicht bestimmt werden. Weder Dursten, noch peroraler Wasserstoß (mit 5% des Körpergewichts) beeinflussen die Veränderungen des extrazellulären Raumes dermaßen, daß daraus auf Flüssigkeitsverschiebung gefolgert werden könnte. Die Untersuchungen bewiesen außerdem, daß die einmalige Bestimmung des

extrazellulären Raumes am ersten Tag ein falsches oder nicht komplettes Bild zeigen kann.

Am zweiten Tag vermindert sich der extrazelluläre Raum im Verhältnis zum 24stündigen erhöhten Wert in signifikanter Weise um etwa 5%. Die

Tabelle I

Veränderungen des extrazellulären Raumes bei Ratten nach Ganzkörperbestrahlung mit 1500 r

Untersuchungszeit nach Strahlenexposition	Zahl der Tiere	Extrazell. Raum (Körpergewicht-%)	p-Wert
Kontrolle + (normal)	18	16,4 ± 1,36	
1 Stunde	7	18,3 ± 0,89	0,20>p>0,10
6 Stunden	5	16,4 ± 2,70	—
12 Stunden	8	13,7 ± 0,24	p < 0,01
24 Stunden	8	19,1 ± 1,31	p < 0,01
48 Stunden	7	14,7 ± 1,21	p < 0,01
72 Stunden	6	9,2 ± 0,30	p ≪ 0,01

Strahlenschädigung des gastrointestinalen Epithels manifestiert sich u. a. in verminderter aktiver und passiver Flüssigkeitsresorption [6]. Der gehinderte gastrointestinale Wasserkreislauf retiniert eine 5% des Körpergewichts entsprechende Menge extrazellulärer Flüssigkeit; als Folge der Anorexie, der verminderten Wasserresorption und der Diurese am ersten Tag ist der Organismus nicht mehr fähig, diesen Verlust zu ersetzen.

Von der 48. Stunde bis zum Tod, also bis zur 72—76. Stunde nimmt der extrazelluläre Raum in gesteigertem Maße — gewissermaßen bezeichnend für das sich parallel entwickelnde gastro-intestinale Syndrom — ab, auf Werte unter 10% des Körpergewichts. Laut des allometrischen extrazellulären Raum—Körpergewicht-Verhältnisses nach GAHLEN und RÖTTGER [8], kann diese Abnahme als absolut betrachtet werden, da das allometrische Verhältnis zwischen Flüssigkeitsraum- und Körpergewichtsabnahme nicht mehr besteht.

Die durchgeführten Experimente demonstrieren die bis zum Exitus 56% erreichende, signifikante Verminderung des extrazellulären Raumes. Laut SWIFT und TAKETA [26] verläuft innerhalb des extrazellulären Raumes die Volumenverminderung des Plasmas (40—60%), zumindest in der letzten Periode parallel mit der Veränderung der interstitiellen Flüssigkeit. MOSER fand im gastrointestinalen Trakt bei mit 1000 r bestrahlten Ratten vor dem Tode eine Flüssigkeitsmenge, die 10% des Körpergewichts entsprach. In unseren Experimenten verminderte sich der extrazelluläre Raum vom 24stündigen Maximalwert gerechnet (d. h. vom Zeitpunkt der Erscheinung der gastro-

intestinalen Symptome) bis zum Tod um eine Flüssigkeitsmenge die 9,9% des Körpergewichts beträgt. Von der 60—65. Stunde an folgte auf die bis dahin langsame Senkung der extrazellulären Raum-Werte ein jäher Abfall, was mit den Beobachtungen von CURRAN [6] in Zusammenhang gebracht werden kann, der zu diesem Zeitpunkt in der Darmwand eine paradoxe Permeabilität für Wasser nachwies.

Die im gastrointestinalen Trakt retinierte Flüssigkeit ist also offensichtlich extrazellulären Ursprungs, obwohl auch intrazellulärer Flüssigkeitsverlust nicht ausgeschlossen werden kann, da die kompensatorische Wechselwirkung der Wasserräume wohlbekannt ist. Es soll nochmals betont werden, daß die extrazellulären Raum-Bestimmungen allein die Frage der pathologischen Dynamik der Wasserräume nicht klären können.

Die Umstände, daß einerseits zwischen Wasser- und Na-Stoffwechsel ein enger Zusammenhang besteht, anderseits — wie dies eigene Untersuchungen [30], sowie Angaben anderer Forscher beweisen —, daß die Na-Ausscheidung beim Strahlensyndrom pathologisch hohe Werte aufweist, lassen auch hinsichtlich der extrazellulären Räume gewisse Folgerungen zu.

Nach letaler Ganzkörperbestrahlung — wie darüber in einer vorangehenden Arbeit bereits berichtet wurde [30] — nimmt nämlich die Na-Ausscheidung zu, im extrazellulären Raum führt die verminderte Na-Konzentration bekanntlich zur Verminderung der Konzentration der übrigen Kationen und auf Grund der Elektroneutralität zur Abnahme der Gesamtelektrolytkonzentration.

Falls Elektrolytkonzentrationsabnahme und Wasserverlust nicht vollkommen parallel verlaufen, verändert sich auch der osmotische Druck der extrazellulären Flüssigkeit, da die in den Körperflüssigkeiten befindlichen Elektrolyte zu 95% für die Aufrechterhaltung des osmotischen Drucks verantwortlich sind.

Die bei Strahlenerkrankungen im extrazellulären Raum verlaufenden Verschiebungen des Wasser- und Na-Haushaltes sind auch hinsichtlich der unter Umständen zustande kommenden osmotischen Druckveränderungen in der extrazellulären Flüssigkeit von Bedeutung.

Die beim Strahlensyndrom beobachtete Verschiebung der Kationen in negativer Richtung, sowie die Einengung des extrazellulären Raumes unterstützen die Annahme, daß primär eine gesteigerte Na-Ausscheidung entsteht und dies zur Störung des gesamten Elektrolyt- und Wasserstoffwechselgleichgewichtes führt.

Auf Grund dieser Annahme ergibt sich die Frage, worin die Ursache der gesteigerten Na-Ausscheidung liegt.

Die auf den Na- und Wasserhaushalt einwirkenden integrierenden Systeme (Hypothalamus, Neurohypophyse, Adenohypophyse) werden annehmbar durch eine Strahlenschädigung von 1500 r lädiert, auch dies kann eine der Ursachen der sich entwickelnden Symptome sein.

Eine nicht zu vernachlässigende Rolle im komplizierten Regulationsmechanismus ist auch den nicht fehlerlos funktionierenden Osmo- und Volumenrezeptoren beizumessen, weiterhin der gestörten Synthese der Hormone, welche die tubuläre Na-Resorption beeinflussen.

Der lädierte Organismus kann aber weder den extrazellulären Raum mit endogenem — in erster Reihe aus Fettgewebeabbau stammenden — Wasser auffüllen, noch die eingeführte Flüssigkeit zurückhalten, wodurch die Katastrophe mit Sicherheit eintrifft. Auf diesem Gebiet warten noch viele Fragen auf Klärung.

LITERATUR

1. Bacq, Z. M., Alexander, P.: Fundamentals of Radiobiology. Pergamon Press, London—New York. 1961.
2. Bálint, P., Hegedüs, A.: Klinische Laboratoriumsdiagnostik. Művelt Nép, Budapest 1955. (Ung.)
3. Bálint, P., Forgács, J.: Kis. Orv. Tud. **8,** 534 (1956).
4. Cannon, W. B.: Traumatic shock. Appleton Ed., New York, 1923.
5. Caster, W. O., Armstrong, W. D.: Radiat. Res. **5,** 189 (1956).
6. Curran, P. F., Solomon, A. K.: J. Gen. Phys. **41,** 143 (1957).
7. Eckel, R. E., Smith, G.: Amer. J. Phys. **38,** 269 (1960).
8. Gahlen, W., Röttger, H.: Klin. Wschr. **36,** 951 (1958).
9. Gömöri, P.: Kollaps und Schock. Orvosképzés, **54,** (1941) (Ung.)
10. Jackson, K. L., Rhodes, R., Entenmann, C.: Radiat. Res. **8,** 361 (1958).
11. Kay, R. E., Entenmann, C.: Amer. J. Phys. **197,** 169 (1959).
12. Kerpel-Fronius, Ö.: Erg. inn. Med. Kinderheilk. **51,** 523 (1936).
13. Kovách, A.: Untersuchungsmethoden der exp. Medizin. Bd. III. Akadémiai Kiadó, Budapest. 1957. (Ung.)
14. Lazarew, N. W., Lazarewa, A.: Strahlentherapie, **23,** 45 (1926).
15. Mertz, D. P.: Klin. Wschr. **38,** 269 (1960).
16. Mertz, D. P., Eppler, F.: Klin. Wschr. **37,** 588 (1959).
17. Moon, V. H.: Shock. Lea-Febiger, Philadelphia, 1942.
18. Pentz, E. I.: Proc. Soc. Exp. Biol. Med. **95,** 3 (1957).
19. Peters, G.: Nebennierenrinden-Inkretion und Wasser-Elektrolythaushalt. G. Thieme, Stuttgart. 1960.
20. Quastler, H.: Radiat. Res. **4,** 303 (1956).
21. Rolf, D., Armstrong, C. M., Steiger, B. W., Audio, M., White, H. C.: Amer. J. Physiol. **196,** 1109 (1959).
22. Rusznyák, I., Földi-Szabó: Physiologie und Pathophysiologie des Lymphkreislaufs. Akadémiai Kiadó, Budapest. 1955. (Ung.)
23. Rusznyák, I., Karády, Hámori: Entstehung d. traumatischen Schocks. Magy. Orv. Arch. **43,** 431 (1942).
24. Schade, F., Menschel, H.: Z. Klin. Med. **96,** 279 (1923).
25. Sheppard, C. W., Darden, E. B.: Fed. Proc. **17,** 148 (1958).
26. Swift, M. N., Taketa, S. T.: Radiat. Res. **8,** 516 (1958).
27. Várteresz, V., Fráter, I., Wald, B.: Kis. Orv. Tud. **8,** 34 (1946).
28. White, H. L., Rolf, D.: Amer. J. Phys. **188,** 151 (1957).
29. Wilde, W. S., Sheppard, C. W.: Proc. Soc. Exp. Biol. Med. **88,** 249 (1955).
30. Zsebők, Z., Petrányi, Gy. jr.: Acta Med. **19,** 137 (1963).

Dr. Zoltán Zsebők
Dr. Győző Petrányi jr. } Budapest, VIII., Üllői út 78, Hungary

TRANSSEPTAL LEFT HEART CATHETERIZATION

By

G. S. KOVÁCS, J. PEPÓ and B. FELKAI

FIRST DEPARTMENT OF SURGERY AND FIRST DEPARTMENT OF MEDICINE, UNIVERSITY MEDICAL SCHOOL, SZEGED

(Received April 10, 1962)

A technique of transseptal left heart catheterization employed with success in 25 out of 28 patients is described in detail. No serious complication was observed. The technique described is simple and safe, and requires no equipment other than that usual in all cardiological laboratories. It yields clear pressure recordings, and permits exact diagnosis of pathological changes in the left heart.

A few characteristic pressure recordings are presented.

The need of exact preoperative cardiological diagnosis has led to the development of a variety of techniques for studying haemodynamic conditions in the chambers of the left heart, such as percutaneous puncture of the left atrium from behind [4], percutaneous puncture of the left atrium from the suprasternal notch [25], percutaneous puncture of the left ventricle by the transthoracic route [5], simultaneous percutaneous puncture of both atria by the transthoracic route [10], left atrial puncture through the bronchoscope [1, 12], retrograde left ventricular catheterization through the exposed left brachial, ulnar, radial [15, 34] or carotid [24] artery, and percutaneous catheterization through the femoral artery [11, 24].

Experience has shown that all these methods have disadvantages and involve hazards [13, 14, 15, 19, 20, 21, 24, 26, 33, 34], and that none of them fulfils all the requisites of interventions of this type: safety, simplicity of technique, absence of discomfort for the patient, the possibility of prolonged intracardial pressure recordings, consistent easy access to both the left atrium and left ventricle, the possibility of combining left heart with right heart catheterization, of injecting contrast material, and of carrying out dye dilution studies.

COURNAND [8] observed, and general experience has since confirmed, how easily a catheter introduced through the great saphenous vein is passed into the left atrium through an atrial septal defect. Prompted by this knowledge COPE [9], in 1959, utilizing a long straight needle introduced inside the lumen of a cardiac catheter, punctured the left atrium through the interatrial septum. Later ROSS and BRAUNWALD [6, 27] used for the same purpose a needle with a curved end; employing various modifications, their method has

been adopted by several workers [2, 17, 30] and used with success for transseptal catheterization of the left atrium.

We have used the method described by STEINHART and ENDRYS [30] since 1960. In this report we present the method and the experience we have so far gained with it

Method

The transseptal puncture needle used by us is a stainless-steel cannula with an outside diameter of 1.2 mm (Fig. 1a). The distal end is shaped with a special curvature (Fig. 1b), and the proximal end terminates in a socket. From socket to tip the needle is 84 cm in length. A metal direction indicator is welded to the socket permitting exact determination of the direc-

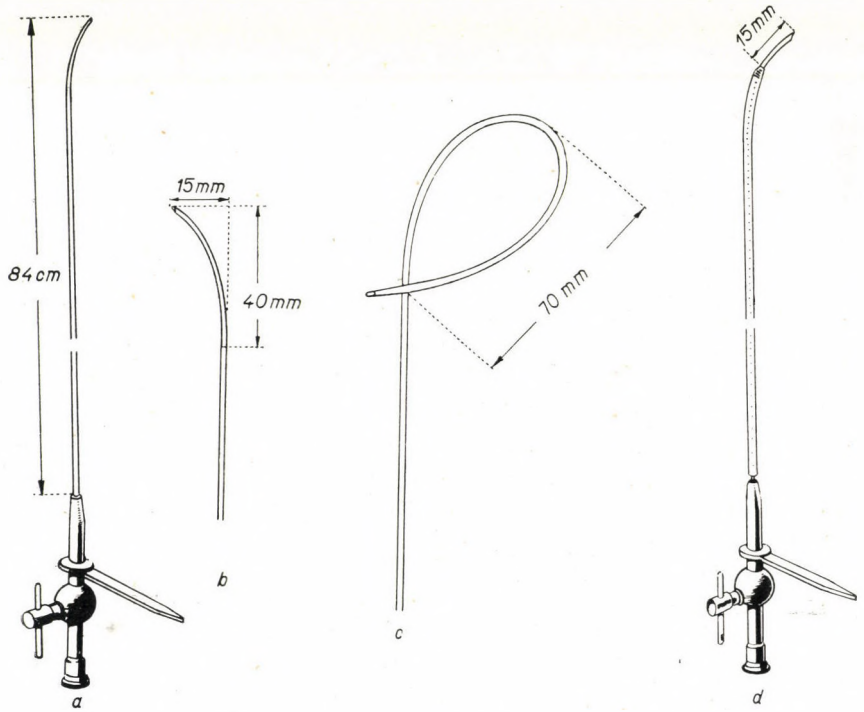


Fig. 1. (*a*) Transseptal puncture needle; (*b*) distal end of needle with curvature; (*c*) curvature of transseptal catheter; (*d*) transseptal catheter passed over the puncture needle

tion in which the tip of the needle points. Below this there is a stop-cock. The socket ends in a closely fitting connecting piece. Although considerably thinner and longer, this needle is similar in shape to that described by ROSS and BRAUNWALD [27].

For the introduction of the needle a yellow-coloured Oedman—Ledin catheter, 82.5 cm in length, with an inside diameter of 1.5 mm, is used. One end, with the tip slightly tapered [23], is curved in the manner illustrated in Fig. 1c; this is essential, for it is the only means to ensure the catheter's passage into the left ventricle. The proximal end is conical to fit the connecting piece. The catheter so prepared is easily slipped over the transseptal puncture needle, of which the distal 15 mm and the tip project from the catheter passed over it in its entire length (Fig. 1d).

The catheter is inserted either percutaneously with SELDINGER's method into the right femoral vein [29], or into the exposed right great saphenous vein. (The latter method is employed when left heart and right heart catheterization are combined.) Straightened with SELDINGER's flexible stylet, the catheter is introduced through the inferior vena cava into the right atrium. The stylet is then removed and replaced by the puncture needle, taking care that by leaving 16 to 18 mm of its proximal end free, its tip be safely concealed inside the catheter.

The needle having been advanced through the lumen of the catheter into the right atrium, transseptal puncture of the left atrium is carried out in the usual manner [30]. (The needle tip concealed in the catheter is positioned at right angles to the interatrial septum: with the aid of the direction indicator on the connector it is rotated toward the right side of

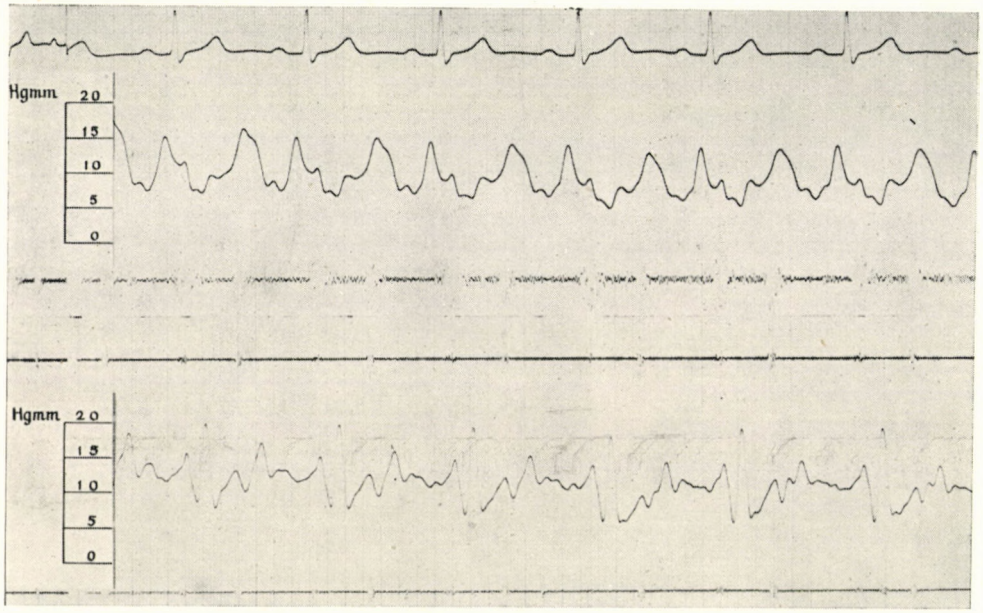


Fig. 2. Normal left atrial pressure recorded on the second channel, and wedge pulmonary artery pressure of the same patient recorded on the fifth channel. On first channel, ECG II.; in the third, fourth and sixth channels, PCG. Recorded with a six-channel "Hellige" Multiscriptor. The left atrial pressure curve is characterized by two peaks: it has two peaks during one action: an atrial contraction and an atrial filling wave

the patient, to the left and posteriorly at about a 45° level between the median and the frontal planes. It is important that rotation should be to the right, else the needle tip might enter the right auricle, whence the aorta may inadvertantly be punctured.) Successful puncture is readily sensed, for the septum is suddenly felt to cease to resist; at the same time, the typical left atrial pressure curve appears on the oscilloscope (Fig. 2), and withdrawal of oxygenated blood confirms the proper location of the needle tip.

When its tip has been safely localized in the left atrium, the needle is fixed and the catheter passed forward over it. The needle is then withdrawn from the catheter, the adapter mounted to its proximal end, and catheterization is carried out in the usual manner.

By rotating the catheter while passing it forward over the needle from 90 to 120° toward the right side of the patient, it is easy to achieve entry into the left ventricle, provided the curvature is of the right shape. To maintain that shape it is important that the catheters be sterilized not by heat but by chemical agents [16]. Excessive regurgitation may render it difficult to enter the left ventricle.

Results

With the method described above, transseptal left heart catheterization has been carried out in 28 patients. The left atrium was successfully entered in all but three cases. The failures occurred in three of our earliest patients, and were due to our lack of experience, and our initial use of transseptal needles with insufficiently pointed tips.

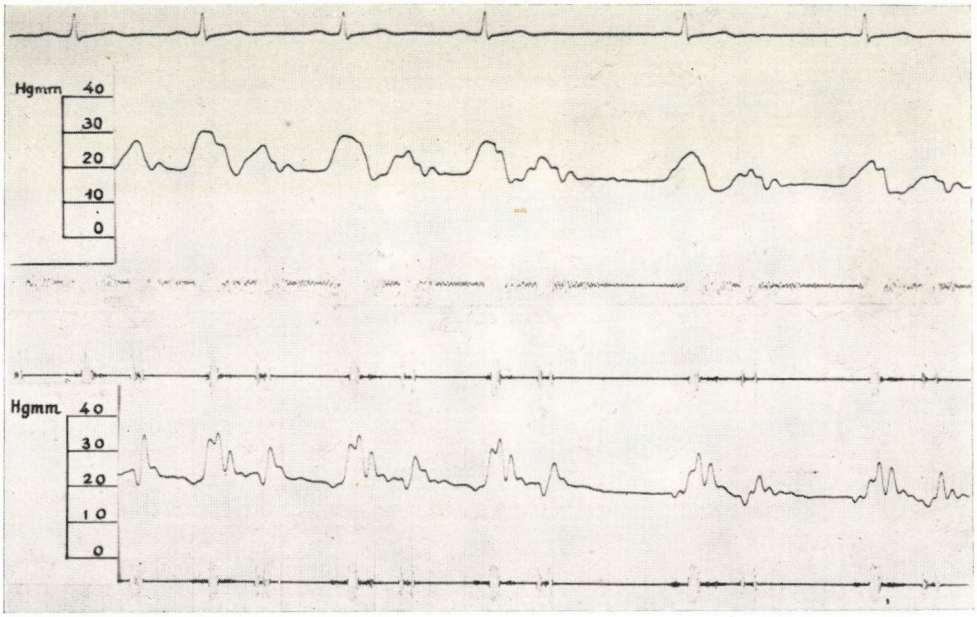


Fig. 3. Left atrial pressure in a patient with pure mitral stenosis recorded on the second channel. On fifth channel, wedge pulmonary pressure artery of the same patient

No unduly severe complications were noted. In one patient with adhesive pericarditis, bigeminia and hypotension developed during left heart catheterization, with temporary loss of consciousness followed by complete recovery within an hour after removal of the catheter. Two patients mentioned a vague and not particularly distressing sensation of pain in the upper part of the chest at the moment the septum was punctured. All the other patients tolerated the intervention without any sign of discomfort. Arrhythmia did not occur either at the time of piercing the septum or of passing the catheter into the left atrium. In some patients a few ventricular extrasystoles were observed when the catheter tip entered the left ventricle, but conspicuously less than are commonly evoked by the catheter tip in the right ventricle on right heart catheterization. To the best of our knowledge, in no case did we inadvertantly puncture the free wall of the atrium or any other nearby structure. No patient

complained of discomfort after catheterization, and all were up and about the same day.

On several occasions exercise tests were performed; on others, dye was introduced into the catheter placed in the left atrium. In some cases, the catheter was allowed to remain in the left atrium for 30 to 40 minutes. In some patients, a second catheter was also introduced (into the aorta, the pulmonary

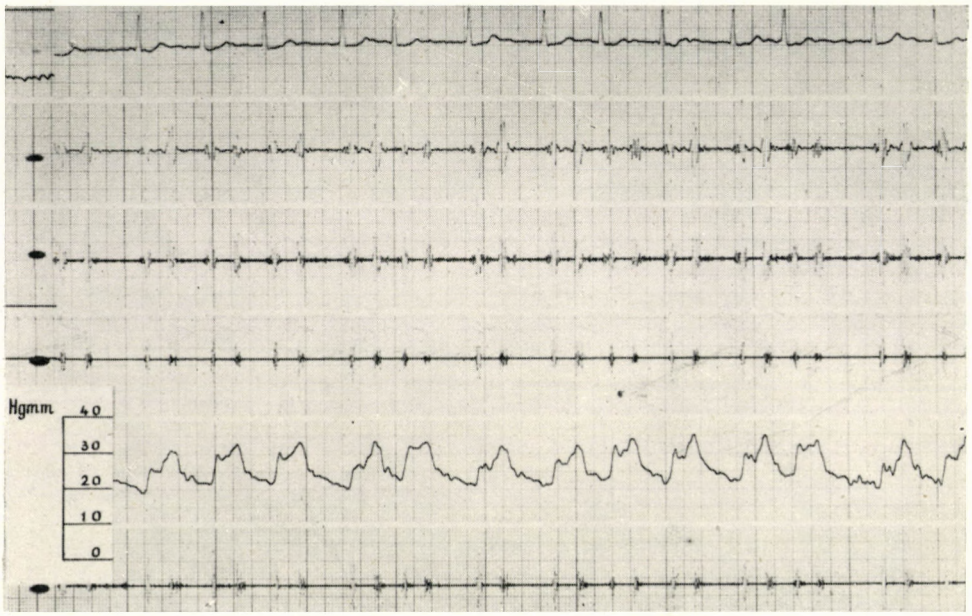


Fig. 4. Left atrial pressure. Combined mitral defect with considerable regurgitation. Atrial fibrillation

artery, etc.) to obtain simultaneous pressure recordings. No complications or discomfort were noted during or after such procedures.

Transseptal left heart catheterization was carried out in patients with mitral defect, actual or suspected aortic defect, and a few cases where right heart catheterization yielded high wedge pulmonary artery pressures that were incompatible with the clinical picture seen. All the pressure curves registered through the catheter were of excellent quality. In patients with mitral defect the catheter was withdrawn from the left into the right atrium, continuously recording the pressure gradient between the two (Fig. 6). In several patients dye was introduced through the catheter into the left atrium and ventricle, respectively, for the determination of regurgitation and cardiac output with the aid of the dye dilution curve registered peripherally. Figs 3 to 9 illustrate some representative curves.

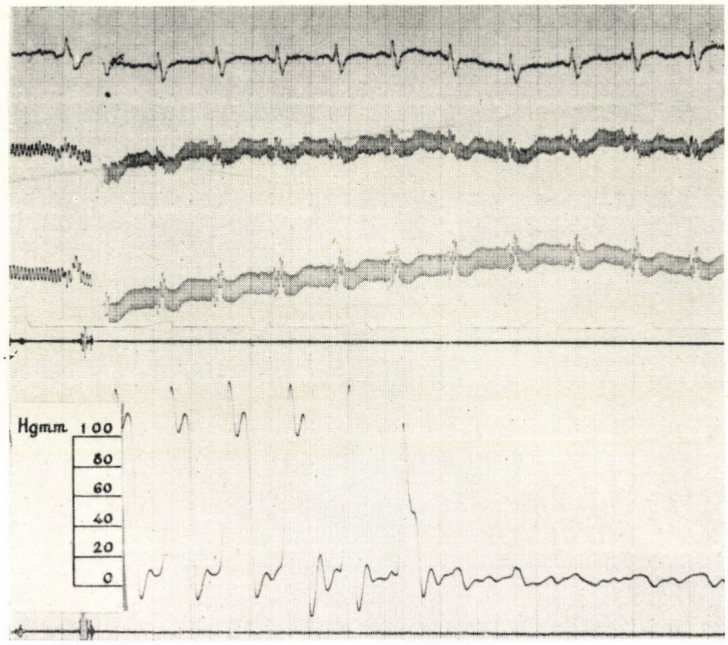


Fig. 5. Withdrawal of catheter from left ventricle into left atrium in a normal subject. No gradient between left ventricular and left atrial diastolic pressures

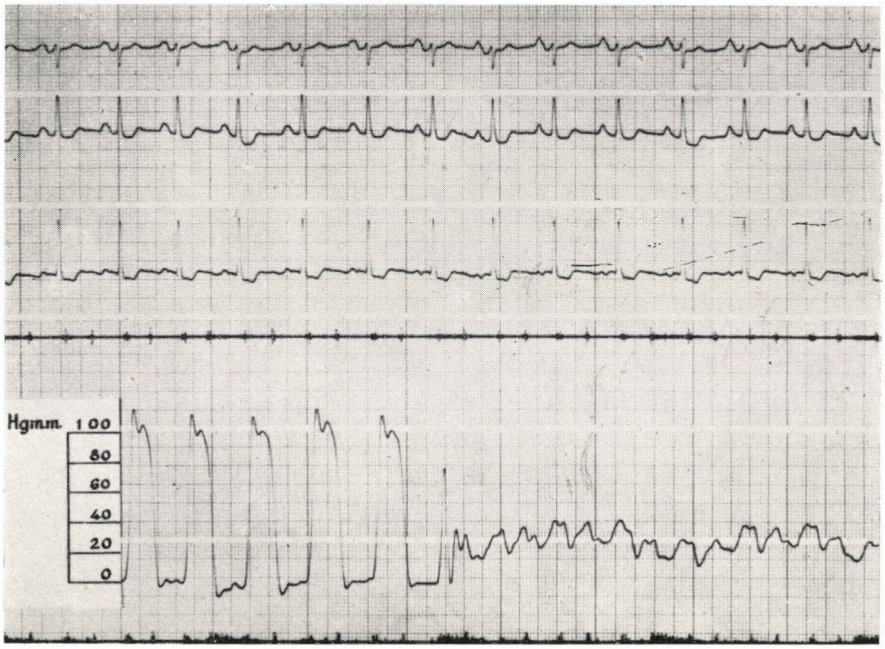


Fig. 6. Withdrawal of the catheter from left ventricle into left atrium in a patient with mitral stenosis. Large gradient

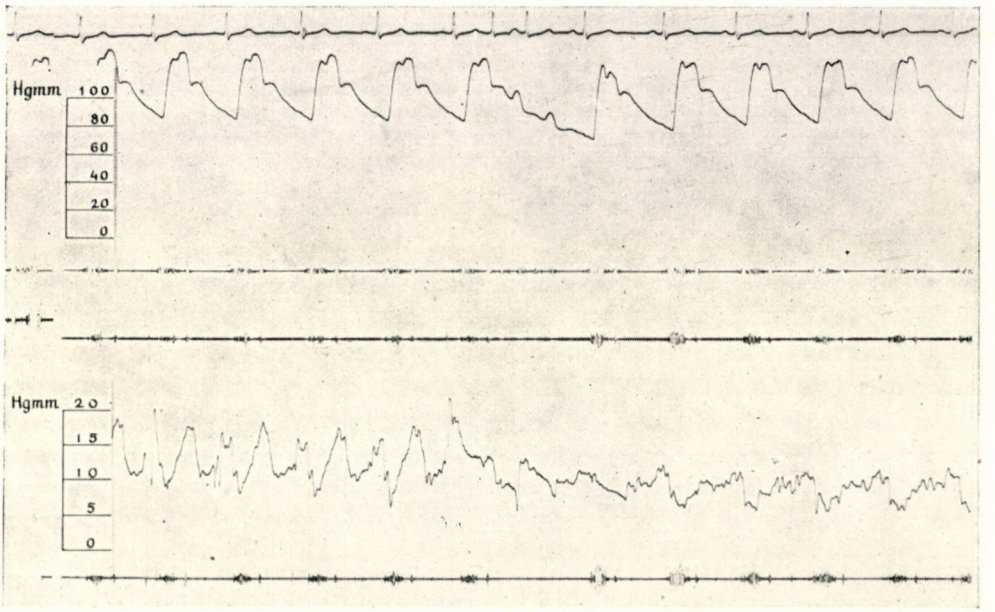


Fig. 7. Withdrawal of catheter from left atrium into right atrium in normal subject (fifth channel). No appreciable pressure gradient. (Aortic pressure curve of the same subject, on second channel.)

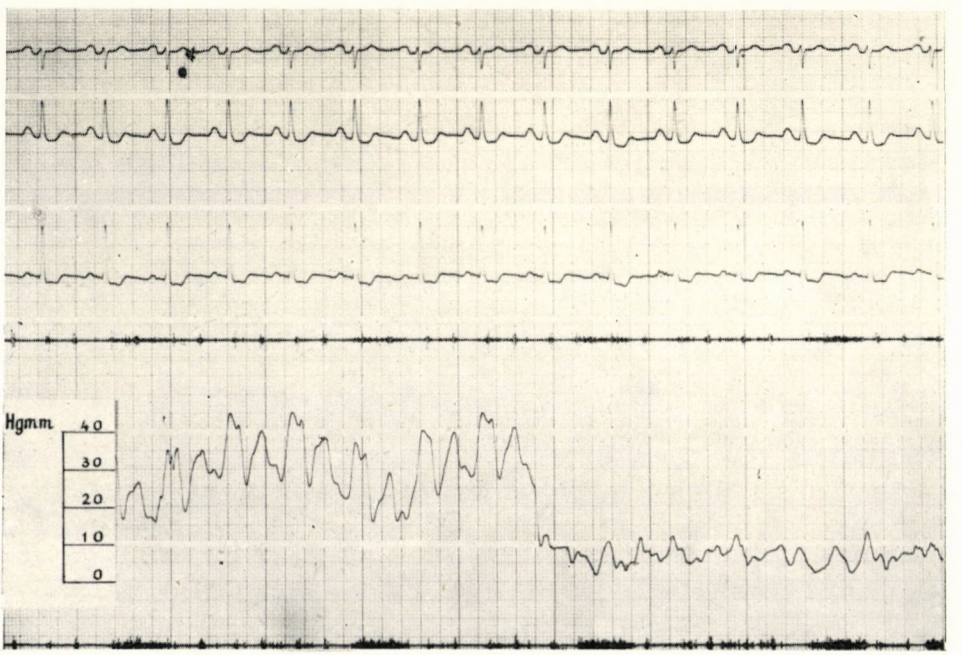


Fig. 8. Withdrawal of catheter from left atrium into right atrium in mitral stenosis. Considerable pressure gradient between the two atria

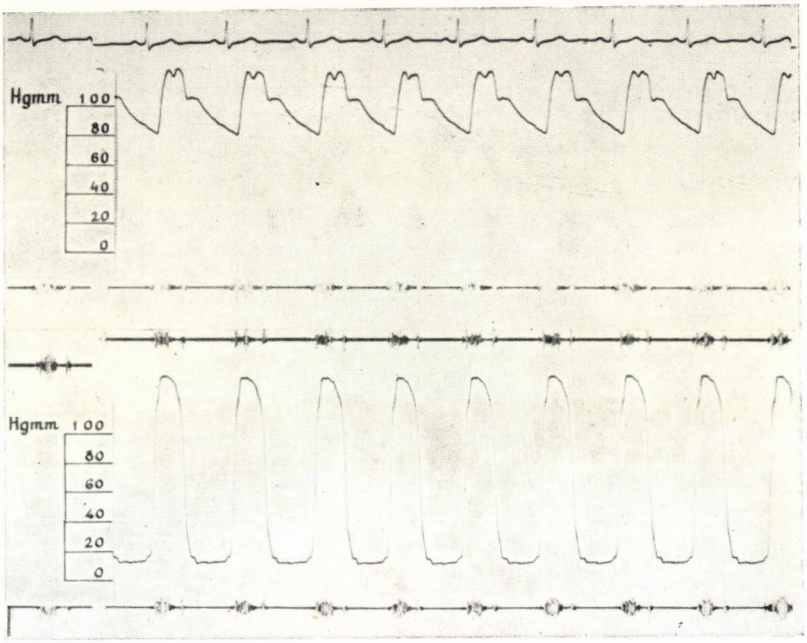


Fig. 9. Left heart catheterization in suspected aortic stenosis. On second channel, aortic pressure; on fifth channel, left ventricular pressure. No essential difference between the two systolic pressures

Discussion

When deciding for surgery in the case of mitral defects it is not always sufficient to know the wedge pulmonary artery pressure. It is often necessary to record the left atrial, and preferably also the left ventricular pressure. This is also a requisite to establishing the haemodynamic conditions in left heart defects. Transseptal left heart catheterization as described in the foregoing, represents a simple and safe method that can be carried out in almost any cardiological laboratory with the usual equipment and, unlike several other left heart catheterization procedures, without the presence of the anaesthetist or bronchologist. As the pressure curves are registered through catheters of a fairly wide lumen, their quality is unobjectionable. Transseptal catheterization is performed with the patient supine and awake; moreover, with the catheter introduced percutaneously into the left atrium, he may even do exercise (ergometry); thus the procedure can be carried out with the patient in a physiological basal state, the same as in right heart catheterization. The method causes no particular discomfort, and provided certain measures of precaution are observed (repeated flushing with heparinized saline solution), the catheter can be left in the left atrium for considerable time without unfavourable consequences. Dyes can be injected into the left atrium through the catheter, and so from the peripherally registered dye dilution curve it is possible to assume

the extent of regurgitation in mitral defects. The catheter permits injection of contrast material into the individual chambers, and the resulting levo-cardio-angiograms are of help in clarifying many diagnostic problems [24, 30]. Transseptal puncture has the additional advantage of lending itself readily to performance in children, where the anatomic conditions preclude other direct puncture methods [2, 28].

Our material now consisting of 28 cases is too small to permit far-reaching conclusions concerning the safety of the method. But if it is reviewed in combination with the more than 800 transseptal punctures reported in the literature, the procedure may be claimed to have involved less hazards than any other method used in left heart catheterization; in fact, it involves no greater risk than does right heart catheterization. To pierce the atrial septum and push the catheter into the left heart are in themselves manipulations incurring no risk, provided due caution is observed, in awareness of the potential danger of embolism in the left heart. No significant arrhythmia at the time of puncture was noted in our cases, nor have any been mentioned in the literature.

The danger of inadvertantly puncturing another structure instead of the septum is greatly reduced by the fact that the tip of the needle is easily directed, and can be used for scanning practically the whole of the septum. Owing to its thinness, the use of our needle will reduce the danger incurred by a possible accidental puncture (for example, of the atrial wall), as long as care is taken not to pass the catheter forward over the needle until the latter's safe location in the left atrium has been verified by the pressure curve on the oscilloscopic screen. We would mention here that in punctures of the left atrium by the posterior percutaneous route performed according to BJÖRK [4], the left atrial wall is always pierced from the outside with a needle considerably thicker than ours; the situation is the same with the transbronchial technique [19]. It seems improbable that piercing the atrial wall from outside should incur less danger than would its accidental puncture from inside.

The question arises whether piercing of the atrial septum may produce a septal defect. ROSS et al. [28] performed the Kr^{85} inhalation test in a number of patients following transseptal puncture, and were unable to demonstrate a persistent left to right shunt in any one of them. At post-mortem examinations of patients who died after operations performed a few days following transseptal puncture, the site of the puncture could hardly be identified [30].

The qualities of, and experiences with, transseptal catheterization compare favourably with all other methods of left heart catheterization, and provide the final considerations needed to assess the best way of treatment. In view of this, the direct percutaneous puncture methods should be abandoned as dangerous and associated with a variety of complications.

Although its relative safety and technical simplicity suggest its use as a diagnostic tool, it must be borne in mind that transseptal left heart catheter-

ization has its strict indications and contraindications. In our opinion it is indicated in every case of postcapillary pulmonary hypertension, in which right heart catheterization has failed to lead to a satisfactory diagnosis. The contraindications are the same as with right heart catheterization; nor do we recommend the technique in cases with a history of arterial embolism suspected to have originated from the left heart. As atrial fibrillation, particularly if of long standing, predisposes to thrombus formation, this also may contraindicate transseptal left heart catheterization. In exceptional cases one should refrain from more than the absolutely essential minimum manipulation, and withdraw the catheter into the right heart as soon as possible after registration of the pressure curve.

REFERENCES

1. ALLISON, P. R., LINDEN, R. J.: Circulation **7,** 669 (1952).
2. BEUREN, A. J., APITZ, J., STOERMER, J.: Z. Kreisl. Forsch. **50,** 644 (1961).
3. BEVEGARD, S., CARLEUS, E., JOHNSSON, B., KARLÖF, I.: Thorax **15,** 299 (1960).
4. BJÖRK, V. O., MALMSTRÖM, G., UGGLA, L. G.: Ann. Surg. **138,** 718 (1953).
5. BROCK, R., MILSTEIN, B. B., ROSS, D. H.: Thorax **11,** 163 (1956).
6. BROCKENBROUGH, E. C., BRAUNWALD, E.: Amer. J. Cardiol. **6,** 1062 (1960).
7. BROCKENBROUGH, E. C., BRAUNWALD, E., ROSS, J. JR.: Circulation **25,** 15 (1962).
8. COURNAND, A., MOTLEY, H. L., HIMMELSTEIN, A., DRESDALE, D., BALDWIN, J.: Amer. J. Physiol. **150,** 267 (1947).
9. COPE, C.: J. thorac. Surg. **37,** 482 (1959).
10. DERRICK, J. R., EGGERS, G. W. N. JR., LEONARD, J. J., PALEY, H. W.: Amer. Heart J. **59,** 442 (1960).
11. DOTTER, C. T., GENSINI, G. G.: Radiology **75,** 171 (1960).
12. FACQUET, J., LEMOIN, J. M., ALHOMME, P., LEFEBOIE, J.: Arch. Mal. Coeur **8,** 741 (1952).
13. FLEMING, H. A., HANCOCK, E. W., MILSTEIN, B. B., ROSS, D. N.: Thorax **13,** 97 (1958).
14. GÖMÖRY, A., SZABÓ, Z.: Orv. Hetil. **100,** 1886 (1959).
15. GRÜNDENAMM, A. M., BOSCH, G. A. C., SCHWANTJE, E. J. M., REIJUS, G. A., VERHENGT, A. P. M.: Amer. J. Cardiol. **6,** 915 (1960).
16. KOVÁCS, G., PEPÓ, J., LÉLEK, I.: Acta Chir. Hung. In press.
17. MCGAFF, C. J., ROVETI, G. C., GLASSMAN, E., ROSS, R. S.: Amer. Heart J. **61,** 161 (1961).
18. MCGUIRE, L. B., HYLAND, J. W., HARRISON, D. C., HAYNES, F. W., DEXTER, L.: Amer. Heart J. **62,** 288 (1961).
19. MORROW, A. G. BRAUNWALD, E., HALLER, J. A. JR., SHARP, E. H.: Circulation **16,** 1033 (1957).
20. MORROW, A. G., BRAUNWALD, E., ROSS, J. JR.: Arch. intern. Med. **105,** 645 (1960).
21. MUSSER, B. G., GOLDBERG, H.: J. thorac. Surg. **34,** 414 (1957).
22. NIXON, P. G. F.: Thorax **15,** 225 (1960).
23. ÖDMAN, P.: Acta radiol. (Stockh.) **52,** 52 (1959).
24. PORSTMANN, W., GEISSLER, W.: Fortschr. Röntgenstr. **91,** 14 (1959).
25. RADNER, S.: Acta med. scand. **148,** 57 (1954).
26. ROMODA, T.: Orv. Hetil. **103,** 306 (1962).
27. ROSS, J. JR., BRAUNWALD, E., MORROW, A. G.: Amer. J. Cardiol. **3,** 653 (1959).
28. ROSS, J. JR., BRAUNWALD, E., MORROW, A. G.: Circulation **22,** 927 (1960).
29. SELDINGER, S. I.: Acta radiol. (Stockh.) **39,** 368 (1953).
30. STEINHART, L., ENDRYS, J.: Fortschr. Röntgenstr. **93,** 753 (1960).
31. SOULIÉ, P., SERVELLE, M., FORMAN, J., OSTY, J., BALEDENT, P., EAGLE, C. C. P.: Arch. Mal. Coeur **00,** 481 (1961).
32. SINGLETON, R. T.: Amer. Heart J. **60,** 879 (1960).
33. TSAKIRIS, A., BÜHLMANN, A., SCHAUB, F.: Cardiologia (Basel) **37,** 386 (1960).
34. ZIMMERMAN, H. A., SCOTT, R. W., BECKER, N. O.: Circulation **1,** 357 (1950).

Dr. Gábor S. KOVÁCS, I. sz. Sebészeti Klinika, Szeged
Dr. János PEPÓ, I. sz. Sebészeti Klinika, Szeged
Dr. Béla FELKAI, I. sz. Belgyógyászati Klinika, Szeged

DYNAMISCHE UNTERSUCHUNG DER JODHALTIGEN AMINOSÄUREN IM SERUM

Von

IRENE R. BALOGH und P. KERTAI

PATHOPHYSIOLOGISCHE ABTEILUNG DES LANDESINSTITUTS FÜR GESUNDHEITSWESEN, BUDAPEST

(Eingegangen am 14. April 1962)

Zur Bestimmung der spezifischen Aktivität der jodhaltigen Stoffe im Blut wird ein einfaches Verfahren beschrieben. Es wurde die Diskrepanz der Verhältnisse der zirkulierenden J^{127} und J^{131} enthaltenden Aminosäuren bestätigt und nachgewiesen, daß diese Diskrepanz in Funktion der Zeit veränderlich ist. Eine Erklärung dieser Veränderungen liegt darin, daß die verschiedenes Jod enthaltenden Aminosäuren in jeweils verschiedenen Zeitpunkten im Kreislauf erscheinen und dort verschieden lang verbleiben.

BLOCK und Mitarb. [1] stellten 1960 fest, daß die Zusammensetzung der im Serum befindlichen J^{127} bzw. J^{131} enthaltenden Aminosäuren sehr verschieden ist. Während J^{131} enthaltende Tyrosinderivate im Serum nicht oder nur in Spuren enthalten sind, machen die J^{127} enthaltenden Tyrosinderivate die Hälfte sämtlicher jodhaltigen Aminosäuren aus. Dies bedeutet also, daß sich die radioaktiven Jodisotope zu einer Analyse der Schilddrüsenhormone im Serum nicht eignen. Vorausgesetzt, daß der Stoffwechsel der stabilen und der radioaktiven Jodisotope identisch vor sich geht, kann obige Feststellung nur auf eine Weise gedeutet werden: Die verschiedenes Jod enthaltenden Aminosäuren erscheinen annehmbar in jeweils verschiedenen Zeitpunkten in der Blutbahn, und der Zeitpunkt ihrer biologischen Periodizität ist ebenfalls verschieden. Das Erscheinen der jodhaltigen Aminosäuren und ihr weiteres Schicksal kann gut verfolgt werden, wenn man ihre spezifische Aktivität in zu verschiedenen Zeitpunkten entnommenen Serumproben bestimmt. Im folgenden werden unsere diesbezüglichen Experimente, die mit J^{131} vorbehandeltem Kaninchenserum ausgeführt wurden, besprochen.

Methode

Vorbereitung des Materials. 12, je 2—2,5 kg wiegenden Kaninchen wurde 400 μC trägerfreies KJ^{131} intravenös verabreicht. Das Präparat wurde vorher papierchromatographisch kontrolliert; 2, 6, 24, 48 und 72 Stunden nach der Einspritzung des Isotops wurde Blut entnommen und die Blutproben unverzüglich zentrifugiert. 4 ml des Serums wurden im Zentrifugenglas mit 0,2 ml 10%igem H_2SO_4 und 8 ml *n*-Butanol gründlich gemischt, zentrifugiert und die obenstehende Flüssigkeit mit einer Pasteur-Pipette abgesaugt. Das Sediment wurde noch zweimal, mit je 5 ml Butanol gewaschen. Die abgesaugten Butanolextrakte wurden mittels 2 *n*-NH_4OH unter Indikatorpapierkontrolle auf pH 7—8 alkalisiert, sodann bei 40° C nicht

übersteigender Temperatur auf 5 ml eingedampft, mit 5 ml Chloroform kräftig durchschüttelt und mit 10 ml 2 *n*-NH_4OH emulgiert. Die Ammoniumphase, die sich von der Butanol-Chloroformfraktion scharf trennte, wurde mit einer Pasteur-Pipette abgesaugt, das Sediment noch zweimal mit je 10 ml 2 *n*-NH_4OH gewaschen. Die vereinigten Ammoniumfraktionen wurden mit Heißluft getrocknet, der Trockenrückstand in 0,5 ml 2 *n*-NH_4OH abermals gelöst und 0,1 ml davon chromatographiert.

Chromatographie

Auf Schleicher-Schüll 2043/a oder Macherey-Nagel 214 Filterpapier (34 × 34 cm) wird die Startlinie 2,5 cm vom unteren Papierrand markiert und das Papier in 3 cm Abständen durch senkrechte Linien in 3 parallele Streifen geteilt. Man pipettiert vorerst auf sämtliche Startpunkte 0,1 ml ammonialkalisches Extrakt, sodann auf die linke Startlinie eine Dijod-

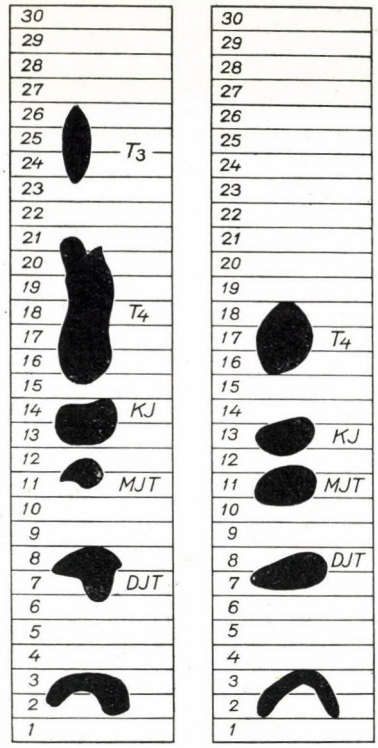


Abb. 1. Papierchromatogramm der aus dem Blut isolierten jodhaltigen Stoffe. Auf dem rechten Papierstreifen ist das Chromatogramm der im Serum befindlichen jodhaltigen Substanzen sichtbar, am linksseitigen das Chromatogramm desselben Serums nach Zugabe von 0,04 μg Thyroxin und 0,04 μg Trijodthyronin. Bezeichnungen s. im Text

tyrosin (DJT), Kaliumjodid (KJ), Thyroxin (T_4) und 3-3′-5′-Trijodthyronin (T_3) enthaltende Standardlösung. Die Laufzeit der aufsteigenden Chromatographie betrug bei Zimmertemperatur 16 Stunden. Lösungsmittel: mit 2 n-NH_4OH gesättigter Butanol. Die wässerige Phase (2 n-NH_4OH) wurde in einem Eindampfungsgefäß in den Zylinder gestellt. Um Dampfsättigung zu erzeugen, stellt man die Lösungsmittel vorangehend für 24 Stunden in den mit drei Gummischichten fest verschlossenen Glaszylinder. Am Ende der Laufzeit wird das Papier getrocknet und die 3 benachbarten Chromatogramme parallel mit der Startlinie in 0,5 cm Abstände geteilt. Nun wird das Chromatogramm in 3 Teile zerschnitten und die Streifen (Extrakt bzw. Extrakt + Standardlösung) auf folgende Weise entwickelt.

Entwicklung des Chromatogramms

Aus jodhaltigen anorganischen und organischen Verbindungen wird auf Einwirkung von Cerisulfat in verschiedenen Verhältnissen Jod freigesetzt, das die $Ce(SO_4)_2$—H_3AsO_3-Reaktion auf bekannte Weise katalisiert. Statt des schwer bewertbaren gelblichweißen Kontrastes entsteht in Anwesenheit von Ferroin-Indikator ein scharfer grün-roter Kontrast, in dem die roten Flecken die jodhaltigen Komponenten anzeigen. Zusammensetzung der zur Entwicklung dienenden Lösung:

3 ml 0,01 *n*-$Ce(SO_4)_2$
4 ml 0,4 n-H_3AsO_3
0,04 ml m/40-Ferroin

Mit Hilfe des Gemisches werden bereits 0,02 μg KJ, sowie 0,04 μg Thyroxin, Dijodtyrosin und Trijodthyronin gut sichtbar: Unter den beschriebenen experimentellen Verhältnissen werden somit sämtliche anorganischen und organischen jodhaltigen Komponenten des Serums sichtbar, mit Ausnahme des in minimalen Mengen vorhandenen Trijodthyronins. Es soll noch bemerkt werden, daß mit dem erwähnten Verfahren die Thyroxin-Fraktion auch geringe Mengen von 3-3′-Dijodthyronin und 3-3′-5′-Trijodthyronin enthält.

Bestimmung der spezifischen Aktivität

Das getrocknete Chromatogramm wird zerschnitten, die Aktivität der einzelnen Papierstückchen mit Hilfe des Szintillationszählers gemessen und prozentual bewertet.

Nach Messung der Aktivität wurden die den einzelnen Fraktionen entsprechenden Papierstückchen vereint und in Supremax-Reagenzgläsern bei 500° C mit Lauge solange verascht, bis die Substanz keine Kohlenstoffteilchen mehr enthielt. Nach Abkühlung wurde die veraschte Substanz mit *n*-HCl neutralisiert, auf 3 ml ergänzt und zur Bestimmung 1 ml verwendet. Zur Bestimmung diente das Mikrojod-Verfahren nach DEMECZKY (Empfindlichkeit: 0,001 μg J). Auf Grund der Menge und Aktivität der einzelnen jodhaltigen Fraktionen wurde die spezifische Aktivität in μg/Impuls/Min ausgedrückt berechnet.

Nach den Paralleluntersuchungen liegt die Fehlergrenze der Methodik unter 10%.

Ergebnisse

In der ersten Versuchsserie wurden die in Butanol löslichen J^{127}- und J^{131}-Fraktionen der 48stündigen Serumproben untersucht und festgestellt, daß zwischen den entsprechenden Fraktionen ein wesentlicher Unterschied besteht.

Abb. 2. zeigt deutlich, daß während das prozentuale Verhältnis von $DJ^{127}T$ und T_3^{127} die Prozentzahl der entsprechenden J^{131} enthaltenden Fraktionen übertrifft, ist der prozentuale Gehalt des Serums an radioaktives Jod enthaltender T_4^{131}-Fraktion höher als der Gehalt an inaktives Jod enthaltender T_4^{127}-Fraktion.

Die Ursache der Diskrepanz liegt entweder in der verschiedenen Geschwindigkeit des Jodisotopeneinbaus in die einzelnen Fraktionen oder darin, daß das Erscheinen bzw. Verschwinden der einzelnen Fraktionen in bzw. aus der Blutbahn zu verschiedenen Zeitpunkten erfolgt. Zur Klärung der Frage wurden die Veränderungen der einzelnen J^{131}-Fraktionen als Funktion der Zeit untersucht.

Wie aus Abb. 3. ersichtlich, sinkt die Menge der KJ^{131}-Fraktion mit der Zeit rapid,die J^{131}-Tyrosin- und T_4^{131}-Fraktionen zeigen vorerst eine anstei-

gende, sodann eine abnehmende Tendenz. Sehr beachtungswert ist die T_3^{131}-Fraktion, die nur in der 24. Stunde im Serum erscheint und in der 72. Stunde noch zunimmt.

Im Gegensatz zu der dynamischen Veränderung der J^{131}-Fraktion zeigt die J^{127}-Fraktion eine scheinbare Stabilität. Die Veränderungen der spezifi-

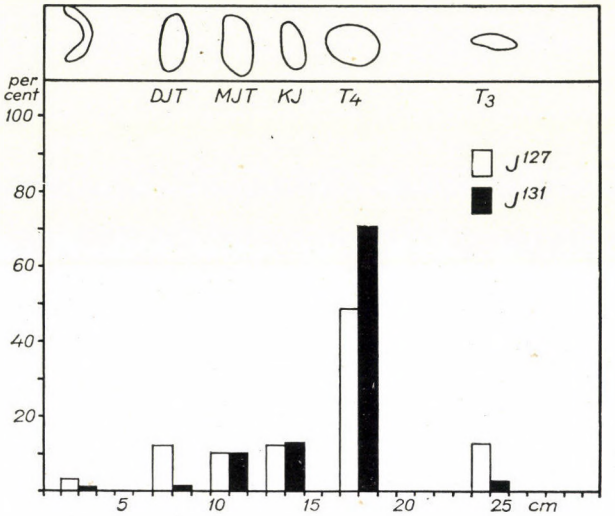


Abb. 2. Die in Butanol löslichen J^{127} und J^{131}-Fraktionen 48stündiger Serumproben. An der Abszisse ist die von der Startlinie berechnete Entfernung der einzelnen Substanzen in cm, an der Ordinate die prozentuale Verteilung der einzelnen Fraktionen sichtbar. Die weißen Kolumnen bezeichnen die inaktiven Jodisotope enthaltenden Fraktionen, die schwarzen die radioaktiven

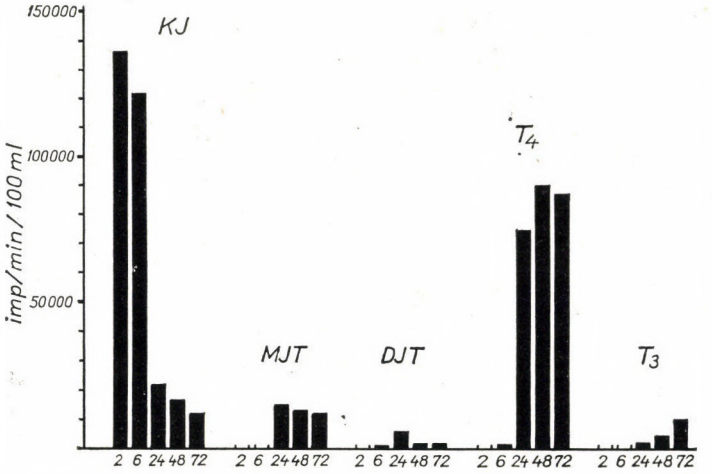


Abb. 3. Aktivitätsveränderungen der J^{131} enthaltenden Fraktionen 2, 6, 24 und 72 Stunden nach Injizierung des Isotops

schen Aktivität gestatten gewisse Folgerungen auf die Reihenfolge des Erscheinens der verschiedenen Schilddrüsenhormone im Blut. Die maximale spezifische Aktivität wurde als 100% betrachtet und die einzelnen Kurven wurden demgemäß dargestellt.

Aus Abb. 4. geht hervor, daß während die spezifische Aktivität des KJ von der 2. Stunde an sukzessiv sinkt, die jodhaltige Tyrosinfraktion das Maxi-

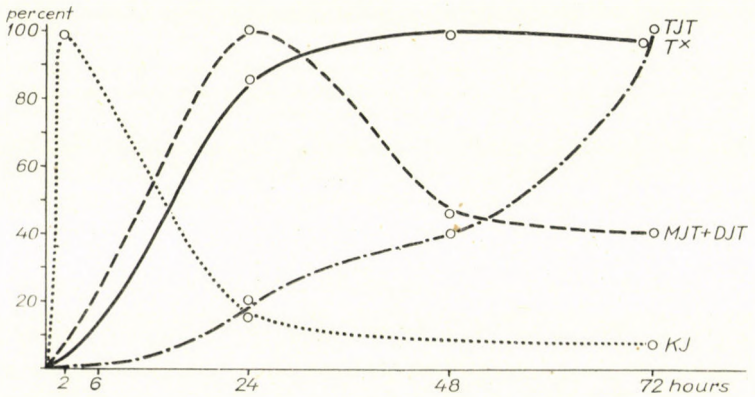


Abb. 4. Veränderungen der spezifischen Aktivität der im Serum befindlichen jodhaltigen Stoffe in Funktion der Zeit. Die spezifische Aktivität der einzelnen Zeitpunkte wurde mit der für 100% gesetzten maximalen spezifischen Aktivität verglichen

mum der spezifischen Aktivität in der 24. Stunde, die T_4-Fraktion in der 48. Stunde erreicht. Die spezifische Aktivität der T_3-Fraktion erreicht lediglich in der 24. Stunde meßbare Werte, der Maximalwert wurde im Rahmen unserer Versuchsserie in der 72. Stunde erhalten.

Besprechung

Die Experimente bestätigten die Diskrepanz der Zusammensetzung der im Blut zirkulierenden J^{127}- und J^{131}-haltigen Aminosäuren und zeigten, daß diese Diskrepanz von der Zeit abhängige Veränderungen aufweist. Diese Veränderungen entstehen dadurch, daß die verschiedenen jodhaltigen Aminosäuren im Kreislauf zu verschiedenen Zeitpunkten erscheinen bzw. sich verschieden lange Zeit in der Blutbahn befinden. Die wichtigste Schlußfolgerung aus den Experimenten ist, daß es prinzipiell keinen einzigen Zeitpunkt gibt, in dem die J^{131} enthaltenden Fraktionen des Serums den tatsächlich bestehenden Verhältnissen der jodhaltigen Bestandteile entsprechen würden.

Bereits 1939 wurde die Frage gestellt, ob das Serum Jodtyrosin enthält. Laut TREVORROW besteht der Jodgehalt des Plasmas aus 30% Thyroxin, 20% anorganisches Jod, 50% Jodtyrosin [2]. Ähnlicherweise fand WILMANNS im

Serum dijodtyrosin- und thyroxinartige Substanzen [3]. Durch die Untersuchung des Verhältnisses der J^{127} enthaltenden Fraktionen mittels Papierchromatographie konnte jedoch die Anwesenheit von Dijodtyrosin nicht nachgewiesen werden [4, 5, 6, 7], um so weniger da Jodtyrosine auch unter den J^{131} enthaltenden Fraktionen nicht nachzuweisen waren [8, 9, 10, 11, 12, 13]. Das aus dem Serum gelegentlich nachgewiesene J^{131}-Dijodtyrosin wurde der schädigenden Einwirkung der radioaktiven Strahlung zugeschrieben. Hinsichtlich unserer Experimente ist die Mitteilung von FEUER von besonderem Interesse. Bei Ratten wurde die spezifische Aktivität der jodhaltigen Aminosäuren im Serum untersucht; weder Mono-, noch Dijodtyrosin konnte nachgewiesen werden, das Verhältnis der T_3- und T_4-Fraktionen zeigte wesentliche Abweichung von den Literaturangaben, und die sog. 3. Substanz, die als Nebenprodukt der Verarbeitung zu betrachten ist, war vorhanden [15].

Zur papierchromatographischen Isolierung von J^{127} enthaltenden Fraktionen wurde von BLOCK, MANDL und WERNER ein neues Verfahren ausgearbeitet [16, 17, 18] und im Laufe ihrer Experimente wurde abermals die Möglichkeit erwogen, ob das Serum Jodtyrosine enthält. DEALE und WHITEHEAD [19] berichteten über ähnliche Beobachtungen. Nachdem die Diskrepanz der J^{131}- und J^{127}-Fraktionen klargelegt wurde, gab BLOCK eine Zusammenfassung der Fehlerquellen, die anläßlich der Isolierungsvorgänge für das Verschwinden der Jodtyrosine verantwortlich waren [1]. Eines dieser Argumente, daß nämlich die Anwendung von Chloroform den Verlust eines Teiles der Jodtyrosine verursachen würde, konnte durch unsere Experimente nicht unterstützt werden.

LITERATUR

1. BLOCK, R. J., WERNER, S. C., MANDL, R. H., ROW, V. V., RA. DI CHEVICH, J.: Arch Biochem. **88,** 98 (1960).
2. TREVORROW, V.: J. biol. Chem. **127,** 237 (1939).
3. WILMANNS, H.: Z. ges. exp. Med. **112,** 1 (1943).
4. MACLAGAN, N. F., BOWDEN, C. H., WILKINSON, J. H.: Biochem. J. **67,** 5 (1957).
5. PIND, K.: Acta endocr. **6,** 263 (1957).
6. WYNN, J.: Arch. Biochem. **87,** 120 (1960).
7. BARNA, S., SZABÓ, G., FEUER, GY. R., BALOGH, I.: Acta med. Acad. Sci. hung. **10,** 339 (1957).
8. TAUROG, A., CHAIKOFF, I. L.: J. biol. Chem. **176,** 639 (1948).
9. TAUROG, A., CHAIKOFF, I. L., TONG, W.: J. biol. Chem. **184,** 99 (1950).
10. LAIDLAW, J. C.: Nature **164,** 927 (1949).
11. ROCHE, J., MICHEL, R.: Physiol. Rev. **35,** 583 (1955).
12. LYBECK, H.: Acta med. scand. **158,** Suppl. 327, 6 (1957).
13. RUEGAMER, W. R., CHODOS, R. B.: Arch. Biochem. **77,** 403 (1958).
14. KENNEDY, T. H., PULVES, H. D.: Aust. J. Exp. Biol. Med. Sci. **9,** 586 (1956).
15. FEUER, GY.: Biochem. J. **73,** 349 (1959).
16. MANDL, R. H., BLOCK, R. J.: Arch. Biochem. **81,** 25 (1959).
17. WERNER, S. C., BLOCK, R. J.: Nature **183,** 406 (1959).
18. BLOCK, R. J., MANDL, R. H., HOWARD, H. W., BAUER, C. D.: Arch. Biochem. **93,** 15 (1961).
19. DEALE, D., WHITEHEAD, J. K.: Clin. Chem. Acta **5,** 150 (1960).

Dr. Irene R. BALOGH, Dr. Pál KERTAI
Budapest IX., Gyáli út 2/6.
Országos Közegészségügyi Intézet, Ungarn

ÜBER DIE WIRKUNG DES OESTRIOLS AUF DEN LIPOIDSTOFFWECHSEL

Von

M. JULESZ, M. B. FRÖHLICH, I. K. LÁSZLÓ, I. TÓTH, G. SZEPESSY und M. A. DÁVID

I. MEDIZINISCHE KLINIK UND ZENTRALLABORATORIUM DER MEDIZINISCHEN UNIVERSITÄT, SZEGED

(Eingegangen am 12. Mai 1962)*

Die Wirkung des Oestriols auf die Gesamtlipoid-, Phosphorlipoid-, Cholesterin- und Cholesterinester- sowie die Alpha- und Beta-Lipoproteinkonzentration des Blutes wurde an sechs Frauen verschiedenen Alters mit verschiedenen Diagnosen untersucht. Das Oestriol übte bei den gegebenen Versuchsbedingungen auf das Gesamtlipoid-, Phosphorlipoid-, Cholesterin- und Cholesterinesterniveau keinen wesentlichen Einfluß aus.

Das Oestriol bewirkte eine signifikante Verminderung des Alpha-Lipoproteingehaltes im Serum und eine Erhöhung der Beta-Lipoproteinkonzentration.

Das Oestriol scheint nicht nur in seiner Wirkung auf das Endometrium, sondern auch bezüglich seines Effektes auf den Lipoidstoffwechsel eine besondere Stelle unter den Oestrogenen einzunehmen.

Die Wirkung der Oestrogene auf den Cholesterinstoffwechsel ist seit langem bekannt (KAUFMANN, 1932); eingehender befaßte man sich mit der Frage aber erst, als der Zusammenhang zwischen Cholesterin und Atherosklerose erkannt wurde. Das Cholesterin ist der Grundstoff der Steroidhormone, und so war zu erwarten, daß gewisse Sexualhormone einen Einfluß auf die Atherosklerose ausübten.

Es fiel auf, daß Koronarsklerose und myokardiale Infarkte bei Frauen vor der Menopause bedeutend seltener vorkommen als bei Männern (GLENDY und Mitarbeiter, 1937; CLAWSON, 1941). Es wurde auch nachgewiesen, daß myokardiale Infarkte bei kastrierten Frauen häufiger sind als bei gleichaltrigen gesunden Frauen (OLIVER und Mitarbeiter, 1954).

Bald stellte sich heraus, daß zur Zeit der geschlechtlichen Reife bei Männern und Frauen unter anderem auch im Lipoid- und Lipoproteingehalt des Serums Unterschiede bestehen (RUSS und Mitarbeiter, 1951; ADLERSBERG und Mitarbeiter, 1956) und der Gedanke, diesen Unterschied mit den Oestrogenen in kausalen Zusammenhang zu bringen, lag auf der Hand. In der Tat hat Oestrogenverabreichung eine Beeinflussung des Lipoidgehaltes des Serums von Männern in »weiblicher« Richtung zur Folge (RUSS und Mitarbeiter, 1955; FURMAN und Mitarbeiter, 1957). Nach den Untersuchungen von OLIVER und Mitarbeitern (1956) setzten die Oestrogene bei Männern mit Hypercholesterinämie und Atherosklerose den Cholesterin- und β-Lipoprotein-Cholesteringehalt des Plasmas herab, während sie den Phosphorlipoid- und α-Lipoprotein-

* Akzeptiert am 18. Sept. 1962.

Cholesterinspiegel erhöhen. Dies wurde mit Bezug auf das Oestron, das 17-β-Oestradiol und verschiedene synthetische Oestrogene nachgewiesen.

Durch die im Serum-Cholesterin- und -Lipoproteinspiegel eintretenden Veränderungen wird nach manchen Autoren (KATZ und Mitarbeiter, 1959; STAMLER und Mitarbeiter, 1957) der klinische Zustand günstig beeinflußt. Die gleichen Autoren betrachteten aber auf Grund ihrer anderweitigen Versuche die klinischen Erfolge als zweifelhaft — obwohl die Oestrogene auch in diesen die Cholesterinkonzentration und den Cholesterin/Phosphorlipoid-Quotienten herabsetzten (STAMLER und Mitarbeiter, 1955, 1959).

Auf den Zusammenhang zwischen Lipoidstoffwechsel und Oestrogenen weisen auch jene Untersuchungen hin, in denen nachgewiesen wurde, daß Männer und Frauen mit myokardialen Infarkten geringere Mengen biologisch aktiver Oestrogene entleeren als gleichaltrige gesunde Individuen (MARMORSTON und Mitarbeiter, 1955, 1957; BERSOHN und Mitarbeiter, 1957). In den letzten Jahren wurde verschiedenerseits festgestellt, daß das gemeinsame Vorkommen von Leberzirrhose und Myokardialinfarkt äußerst selten ist (KWAAN und Mitarbeiter, 1956, GRANT und Mitarbeiter, 1959, HOWELL und Mitarbeiter, 1960, RUEBNER und Mitarbeiter, 1961). KWAAN und Mitarbeiter (1956) dachten auf die Möglichkeit, daß die Leberzirrhose durch Verkleinerung des Atheroms in den Koronarien oder infolge der gesteigerten Fibrinolyse gegen Infarkt schützt. Auf einen Zusammenhang zwischen Leberzirrhose und den hohen Oestrogenspiegel wurde jedoch nicht hingewiesen.

Hieraus wird verständlich, daß in letzter Zeit mehrere Forscher nach Oestrogenen suchen, welche das Serumcholesterinniveau herabsetzen, ohne eine feminisierende Wirkung auszuüben. Die Oestrogenbehandlung von Männern geht nämlich mit überaus unangenehmen Feminisationserscheinungen, Herabsetzung oder Verlust der Libido und Gynäkomastie einher. Das neue synthetische Oestrogen Manvene (3-Methoxy-16-alpha-methyl-oestra-1,3,5(10)trien-16-beta, 17-beta-diol) hat bei Menschen einen günstigen Einfluß auf den Cholesterinstoffwechsel entfaltet und den Rattenuterus sozusagen unbeeinflußt gelassen, sich bei Menschen aber als stark oestrogen-wirksam erwiesen (COHEN und Mitarbeiter, 1959). Ein weiteres neues Oestrogen, das Präparat SC 8246 (16-alpha-Cholesteron-3-methyl-aether) hat RIVIN (1959) ausprobiert. Bei Männern, die akute myokardiale Infarkte überstanden hatten, bewirkte es 6—8 Monate verabreicht in einem beträchtlichen Teil der Fälle eine Erhöhung des Alpha/Beta-Lipoproteinquotienten, ohne jedoch den Serumcholesteringehalt herabzusetzen. Abgesehen von der geringgradigen Gynäkomastie ging die auf die Lipoproteine entfaltete Wirkung nicht mit unangenehmen Nebenwirkungen einher.

Die angeführten Angaben bedeuten nicht unbedingt, daß der die Koronarienkrankheit hemmende Effekt der Oestrogene mit der auf die Lipoide bzw. Lipoproteine entfalteten Wirkung zusammenhängt, es können auch zahlreiche

andere Faktoren — eine Beeinflussung anderer Hormone, der Psyche, Gefäßpermeabilität und des Leberstoffwechsels — in Frage kommen. Dessenungeachtet ist aber auch dann die weitere Suche nach Oestrogenen, die einen ausgesprochenen Einfluß auf den Fettstoffwechsel ausüben, gleichzeitig aber nicht feminisieren, angezeigt.

Der Gedanke, auch das Oestriol in dieser Hinsicht zu prüfen, lag auf der Hand. Seine Wirkung ist bei Menschen und Nagern hauptsächlich auf Cervix, Vagina und Vulva gerichtet (PUCK und Mitarbeiter 1956, PUCK 1957, PUCK und Mitarbeiter 1957, PUCK 1958), während es das Endometrium kaum beeinflußt. Da es bei Frauen im Klimakterium keine Blutungen verursacht, ist es vorzüglich zur Behandlung klimakterischer Beschwerden geeignet. Über die Wirkung des Oestriols auf den Lipoidstoffwechsel haben wir in der uns zugänglichen Literatur keine Angaben gefunden.

Methodik

Die Probanden waren sechs Frauen verschiedenen Alters (19—52 Jahre). 1. Inoperabiles Uteruscarcinom, Alter 50 Jahre. 2. Schizophrenie, Alter 52 Jahre. 3. Stein-Leventhalsyndrom, 34 Jahre. 4. Idiopathischer Hirsutismus, 19 Jahre. 5. Klimakterium, 48 Jahre. 6. Inaktive Tuberkulose, 41 Jahre.

Eine Woche vor der Behandlung wurde folgende quantitative Diät angesetzt: 40 g Gesamtkalorien/kg Körpergewicht in folgender Aufteilung: 50% Kohlenhydrat, 35% Fett und 15% Eiweiß, welche Zusammensetzung der bei uns üblichen Ernährungsweise entspricht. Nach der einwöchigen Vorperiode wurden bei der gleichen Diät eine Woche lang täglich 250 μg Oestriol (O v e s t i n, Organon) verabreicht. Die einwöchige Behandlung bewirkt nach den mit anderen Oestrogenen gemachten Erfahrungen eine ausgesprochene Beeinflussung des Fettstoffwechsels (DICZFALUSY und Mitarbeiter 1961). Eine Woche nach dem Absetzen der Oestrioldarreichung wurde die quantitative Diät fortgesetzt. Sowohl in der Vor- als auch in der Versuchs- und Nachperiode wurden — immer an zwei gleichen Wochentagen — aus Blutproben bei nüchternem Magen Gesamtfett, Phosphorlipoid, Gesamtcholesterin, Cholesterinester sowie die Serumlipoproteinfraktionen bestimmt.

Die Serum-Gesamtlipoidbestimmungen wurden nach der Methode von LINDHOLM (1956) vorgenommen. Nach der Extraktion der Serumlipoide mit Alkohol-Azeton wurde das Alkohol-Azetongemisch abdestilliert und der Rückstand in Petroläther aufgenommen, worauf die Lipoide in Lösung gingen, während die als Verunreinigungen vorhandenen Salze ungelöst blieben. Nach Abdampfen des Petroläthers wurde die Lipoidmenge gravimetrisch bestimmt. Die Normalwerte betragen 600—1500 mg%.

Die Bestimmung des Serum-Phosphorlipoids geschah nach dem Verfahren von LINDHOLM. Die Phosphorlipoide wurden nach Ausfällung der Eiweiße mit Alkohol-Azeton extrahiert, im Kjeldahl-Kolben zerstört und anschließend der Phosphorgehalt bestimmt. Nach Multiplizieren der Lipoid-Phosphorwerte mit 25 wurde der Phosphor-Lipoidgehalt in mg% umgerechnet. Normalwerte: 150—410 mg%.

Die Gesamtcholesterinbestimmung erfolgte nach der Methode von ZAK (ZAK und Mitarbeiter, 1954, 1957), die darauf beruht, daß das Cholesterin in essigsaurem Medium mit schwefelsaurem Ferrichlorid eine der vorhandenen Cholesterinmenge proportionale intensiv violett-rote Farbe gibt.

Zur Bestimmung des Cholesterinesters bedienten wir uns ebenfalls der ZAKschen Methode. Nach Ausfällen des freien Cholesterins mit Digitonin wurde das Cholesterin der obigen Farbreaktion unterzogen, die dabei erhaltene Farbintensität von den Gesamtcholesterinwerten abgezogen und so die Cholesterinestermenge erhalten.

Die Lipoproteinfraktionen wurden elektrophoretisch — in Veronalnatrium-Natriumazetat-Pufferlösung von pH 8,6 bei 200 V Spannung und einer Stromintensität von 2 mA pro Streifen — getrennt (GRASSMANN und Mitarbeiter 1952). Nach etwa 18-stündigem Lauf wurden die Papierstreifen mit Sudanschwarz B gefärbt (SWAN, 1953). Die quantitative Auswertung der einzelnen Fraktionen geschah mit dem Beckman-Spinco Verfahren. Normale Verteilung der Alpha- und Beta-Lipoproteinfraktionen: Alpha = 20—30%, Beta = 70—80%.

Ergebnisse

Die mit der Studentschen »*t*«-Probe bewerteten Ergebnisse sind in Tabellen 1, 2 und 3 zusammengefaßt. Die biometrische Analyse geschah auf Grund der Berechnung der Unterschiedsmittelwerte.

Tabelle

Veränderung des Gesamtlipoid- und Phosphorlipoidgehaltes auf Wirkung der Oestriolbehandlung

		Vor der Behandlung	Während der Behandlung		Nach der Behandlung	
			am 4. Tage	am 8. Tage	am 11. Tage	am 15. Tage
Gesamtlipoid mg%	Durchschnitt	782,4	866,3	760,5	831,3	829,3
	Veränderung	—	±73,7 ±33,9	−31,8 ±39,1	+39,0 ±59,7	+37,0 ±64,8
Phosphorlipoid mg%	Durchschnitt	224,5	233,5	232,2	265,3	230,7
	Veränderung	—	+ 9,7 ±13,2	+ 8,2 ±19,2	+41,5 ±40,5	+ 6,8 ±16,4
Wahrscheinlichkeit	Gesamtlipoid		$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$
	Phosphorlipoid		$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$

Tabelle 1 veranschaulicht die Veränderung der Gesamtlipoid- und der Phosphorlipoidwerte während und nach der Oestriolbehandlung gegenüber den Ausgangswerten. Die vor der Behandlung erhaltenen Daten stellen den Mittelwert der bei den 6 Kranken zu zwei verschiedenen Zeitpunkten gemessenen Werte in der ersten Woche der Diät dar, während die im Laufe der Behandlung bzw. nach Absetzen derselben registrierten in jedem Falle die Durchschnittswerte der bei den 6 Frauen gleichzeitig vorgenommenen Bestimmungen bedeuten. Durch Vergleich derselben mit den Ausgangswerten wurde die Veränderung bzw. die Signifikanz der Veränderungen berechnet. Auch die Berechnung der übrigen Parameter erfolgte unter Zugrundelegung dieses Verfahrens.

Nach Tabelle 1 waren die Veränderungen im Gesamtlipoid- und im Phosphorlipoidgehalt belanglos: p war in sämtlichen Fällen $> 0{,}05$.

Tabelle 2 zeigt die Veränderungen des Serumcholesterin- und -Cholesterinesterniveaus gegenüber den Ausgangswerten während und nach der Oestriolbehandlung. Die Berechnung erfolgte auf die gleiche Weise wie in Tabelle 1. Eine Signifikanz ergab sich weder hinsichtlich der Veränderungen im Serumcholesteringehalt, noch bezüglich des Cholesterinesters: $p > 0{,}05$.

Tabelle 2

Veränderung des Cholesterin- und Cholesterinestergehaltes auf Wirkung der Oestriolbehandlung

		Vor der Behandlung	Während der Behandlung		Nach der Behandlung	
			am 4. Tage	am 8. Tage	am 11. Tage	am 15. Tage
Cholesterin mg%	Durchschnitt	259,4	258,7	263,5	244,7	241,5
	Veränderung	—	+ 3,5 ±15,8	+10,8 ±11,0	−10,2 ±20,0	−13,7 ±18,2
Cholesterinester mg%	Durchschnitt	187,6	191,8	185,7	172,0	172,5
	Veränderung	—	+ 5,5 ±16,3	− 2,3 ±14,6	−16,0 ±18,8	−15,5 ±17,0
Wahrscheinlichkeit	Cholesterin		$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$
	Cholesterinester		$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$	$p > 0{,}05$

Tabelle 3

Veränderung des Lipoproteingehaltes auf Wirkung der Oestriolbehandlung

		Vor der Behandlung	Während der Behandlung		Nach der Behandlung	
			am 4. Tage	am 8. Tage	am 11. Tage	am 15. Tage
α-Lipoprotein %	Durchschnitt	22,2	19,8	14,8	17,7	18,9
	Veränderung	—	−2,5 ±2,0	−9,1 ±2,3	−4,6 -±4,1	−4,6 ±6,3
β-Lipoprotein %	Durchschnitt	77,7	80,4	85,4	82,4	81,0
	Veränderung	—	+4,0 ±2,0	+8,7 ±2,3	+4,7 ±4,2	+4,4 ±6,4
Wahrscheinlichkeit	α-Lipoprotein		$p > 0{,}05$	$p < 0{,}01$	$p > 0{,}05$	$p > 0{,}05$
	β-Lipoprotein		$p > 0{,}05$	$p < 0{,}01$	$p > 0{,}05$	$p > 0{,}05$

Ähnlicherweise wurden die Änderungen der Alpha- und Beta-Lipoproteine vor und nach der Oestriolbehandlung verfolgt. Aus Tabelle 3 erhellt, daß am 8. Tage der Behandlung das Alpha-Lipoprotein signifikant vermindert und das Beta-Lipoprotein dementsprechend signifikant erhöht war.

Besprechung

In den vorliegenden Versuchen wurde die Wirkung des Oestriols an Frauen erprobt, die an einer der normalen Ernährungsweise entsprechenden quantitativen Diät gehalten wurden. Nach den weiter oben angeführten Literaturangaben nehmen die Autoren im allgemeinen einen Zusammenhang zwischen dem bei Frauen beobachteten gewissen Schutz gegen Myokardialinfarkt und den Oestrogenen an. Den Umstand, daß bei Männern mit Leberzirrhose ein Myokardialinfarkt seltener vorkommt, erklären wir — zumindest teilweise — mit dem infolge der Leberläsion vorhandenen höheren Oestrogenblutspiegel. Das Oestriol verfügt — wenigstens bei Frauen — über manche biologische Vorteile gegenüber Oestron und Oestradiol, die eine Untersuchung seines Einflusses auf den Stoffwechsel unbedingt indiziert erscheinen lassen.

Die Versuche wurden an Frauen teils vor und teils nach der Menopause vorgenommen und ergaben, daß eine Woche hindurch verabreichte Oestriolgaben von 250 μg pro Tag bei Frauen im Klimakterium keine Blutungen und bei den in der geschlechtlichen Aktivität befindlichen keine Störungen im Zyklus verursachen.

Diese Versuche beweisen, daß es sich beim Oestriol um eine hinsichtlich des Fettstoffwechsels aktive Substanz handelt, welche — ohne den Gesamtlipoid-, Phosphorlipoid-, Cholesterin- und Cholesterinestergehalt des Blutes wesentlich zu beeinflussen — die Alpha-Lipoproteinkonzentration signifikant herabsetzt und den Beta-Lipoproteingehalt erhöht.

Es fragt sich nun, ob diese Wirkung im Einklang mit den Literaturangaben über andere Oestrogene steht. Ist es möglich, daß ohne Änderungen des Serumcholesterinniveaus im Lipoproteingehalt des Serums eine wesentliche Änderung zustande komme? Aus den Versuchen von Oliver und Mitarbeitern (1956) wissen wir, daß Oestrogene bei hypercholesterinämischen Männern das Beta-Lipoproteinniveau herabsetzen und den Alpha-Lipoproteingehalt erhöhen. Dies steht nicht im Einklang mit unseren Untersuchungsergebnissen. Hier ist aber in Betracht zu ziehen, daß wir unsere Untersuchungen an Frauen vorgenommen hatten. Die obigen Autoren beobachteten aber auch andere Abweichungen im Fettstoffwechsel, und zwar eine Verminderung des Cholesterin- und einen Anstieg des Phosphorlipoidgehaltes im Serum. Unsere Befunde stimmen insofern mit den Angaben von Rivin (1959) überein, als auch wir fanden, daß die quantitative Änderung der Lipoproteine nicht unbedingt mit einer Änderung des Serumcholesterinniveaus einhergehen muß. Rivin fand, daß bei Männern mit Myokardialinfarkt das synthetische Oestrogen SC 8246 den Cholesteringehalt in der Mehrzahl der Fälle nicht herabsetzte, obzwar der Alpha/Beta-Lipoproteinquotient erhöht war.

Was die Frage betrifft, ob die Schutzwirkung der Oestrogene gegen Koronarthrombose mit dem auf die Lipoproteine entfalteten Effekt zusammen-

hängt, gehen die Meinungen auseinander. Die Annahme eines solchen Zusammenhanges hat die sehr umfangreichen Untersuchungen ausgelöst, in denen man bestrebt war, den »ungünstigen« Lipoidstoffwechsel der Männer mit Oestrogenen zu korrigieren. Die therapeutischen Bemühungen haben sich als derart »heroisch« erwiesen, daß mitunter die Gynäkomastie eine Mammaablation nötig machte.

Der Einfluß des Oestriols auf die Lipoproteinfraktionen bei Frauen wird durch unsere Versuche mit großer Wahrscheinlichkeit bestätigt. Die Richtung der Aktivität weicht aber von der der übrigen Oestrogene ab. Das Oestriol scheint nicht nur in seiner Wirkung auf das Endometrium, sondern auch — was seine Wirkung auf den Lipoidstoffwechsel betrifft — eine besondere Stelle unter den Oestrogenen einzunehmen. Es fragt sich, ob es auch den Lipoidstoffwechsel der Männer in ähnlicher Weise beeinflußt. Seine Ausprobierung an Männern halten wir jedenfalls für angezeigt.

SCHRIFTTUM

1. Adlersberg, D., Schaefer, L. E., Steinberg, A. G., Wang, Chun-I: J. Amer. med. Ass. **162,** 619 (1956).
2. Bersohn, I., Oelofse, P. J.: S. Afr. med. J. **31,** 1172 (1957).
3. Clawson, B. J.: Amer. Heart J. **22,** 607 (1941).
4. Cohen, W. D., Higano, N., Robinson, R. W.: In Hormones and Atherosclerosis, Hoeber, New York, 1959 p. 443.
5. Diczfalusy, E., Lauritzen, Ch.: Oestrogene beim Menschen. Springer, Berlin, 1961.
6. Furman, R. H., Howard, R. P.: Ann. intern. Med. **47,** 969 (1957).
7. Glendy, R. E., Levine, S. A., White, P. D.: J. Amer. med. Ass. **109,** 1775 (1937).
8. Grant, W. C., Wasserman, F., Rodenski, P. L., Thomson, R. V.: Ann. intern. Med. **51,** 774 (1959).
9. Grassmann, W., Hannig, K.: Hoppe-Seylers Z. physiol. Chem. **290,** 1 (1952).
10. Howell, W. L., Manion, W. C.: Amer. Heart J. **60,** 341 (1960).
11. Katz, L. N., Stamler, J., Pick, R.: Nutrition and Atherosclerosis. Lea and Febiger, Philadelphia, 1959.
12. Kaufmann, C.: Zbl. Gynäk. **56,** 2058 (1932).
13. Kwaan, H. C., Mc Fadzean, A. J. S., Cook, J.: Lancet. **1,** 132 (1956).
14. Lindholm, H.: Scand. J. clin. lab. Invest., 8, Suppl. **23** (1956).
15. Marmorston, J., Hoffman, O., Sobel, K., Starr, P.: Minn. Med. **38,** 800 (1955).
16. Marmorston, J., Lewis, J. J., Bernstein, J. L., Sobel, H., Kuzma, C., Alexander, R., Magidson, D., Moore, F. J.: Geriatrics, **12,** 297 (1957).
17. Oliver, M. F., Boyd, G. S.: Amer. Heart J. **47,** 348 (1954).
18. Oliver, M. F., Boyd, G. S.: Circulation, **13,** 82 (1956).
19. Puck, A.: Münch. med. Wschr. **99,** 1505 (1957).
20. Puck, A.: Geburtsh. u. Frauenheilk. **18,** 998 (1958).
21. Puck, A., Hübner, K. A.: Acta endocr. (Kbh) **22,** 191 (1956).
22. Puck, A., Korte, W., Hübner, K. A.: Dtsch. Med. Wschr. **82,** 1864 (1957).
23. Rivin, A. U.: Metabolism, **8,** 704 (1959).
24. Ruebner, B. H., Miyai, K., Abbey, H.: Lancet, **2,** 1435 (1961).
25. Russ, E. M., Eder, H. A., Barr, D. P.: Amer. J. Med. **11,** 468 (1951).
26. Russ, E. M., Eder, H. A., Barr, D. P.: Amer. J. Med. **19,** 4 (1955).
27. Stamler, J., Katz, L. N., Pick, R., Rodbard, S.: Progr. Hormone Res. **11,** 401 (1955).
28. Stamler, J., Pick, R., Katz, L. N., Kaplan, B. M.: In Hormones and Atherosclerosis. Academic Press, New York 1959.
29. Stamler, J., Pick, R., Katz, L. N., Kaplan, B., Pick, A.: Circulation, **16,** 940 (1957).
30. Swan, B.: Studies on Blood Lipids. Scand. J. clin. lab. Invest. 5. Suppl. 9 (1953).

31. Zak, B., Dickenman, R. C., White, E. G., Burnett, H., Chernay, P. J.: Amer. J. clin. Path. **24**, 1307 (1954).
32. Zak, B., Luz, D. A., Fischer, M.: Amer. J. med. Technol. 283, 1957.

Dr. M. Julesz
Dr. M. B. Fröhlich
Dr. I. K. László
Dr. I. Tóth
Dr. G. Szepessy
Dr. M. A. Dávid

Szeged, I. Med. Klinik. Ungarn

THYROID FUNCTION DURING TREATMENT WITH THE ERGOT DERIVATIVE LYSERGIC ACID BUTANOLAMIDE

By

L. Szántó, Alice L. Reviczky and T. Grynaeus

SECOND DEPARTMENT OF MEDICINE AND BALNEOLOGICAL RESEARCH INSTITUTE, STATE INSTITUTE OF RHEUMATOLOGY AND BALNEOLOGY, BUDAPEST

(Received May 21, 1962)

The effect of lysergic acid butanol amide (MLAB), a serotonin antagonist, on thyroid function has been studied, on the basis of ^{131}I incorporation into the thyroid, of the iodine content of the thyroid and serum as determined by paper chromatographic analysis, of the interaction between serotonin and MLAB added to rat sera incubated in vitro in Krebs—Ringer phosphate buffer, as well as of results obtained in human subjects. In every parameter examined, MLAB proved to increase thyroid activity:

1. The incorporation of ^{131}I into the thyroid increased.
2. The free T_4 content of the rat thyroid and the conversion rate increased.
3. The T_4 output of the thyroid increased.
4. Peripheral deiodination increased both in vivo and in vitro.
5. Indirect studies (MLAB + methylthiouracil, MTU) yielded results indicative of an increased TSH secretion.
6. Histological examinations showed a gradual development of thyroid hyperfunction.
7. In human experiments involving normal subjects and patients with myxoedema, signs of increasing thyroid activity were demonstrated.

It has been suggested that MLAB interferes with the function of the hypothalamic-pituitary-thyroid-periphery system at three points, *viz.*

it increases peripheral deiodination;

it stimulates thyroid function directly; and

it stimulates thyroid function indirectly, through the hypothalamic-pituitary system.

The discovery and therapeutical use of the thiouracil preparations (Astwood, MacKenzie, 1, 2) represented a significant advance in thyroid pathology. The investigations involving the use of antithyroid agents have elucidated the single phases of thyroid hormone synthesis. The thyroid hormone supply of the organism depends on the synthesis and metabolization of these hormones. While the problems of synthesis have been studied for more than two decades, those of degradation could be investigated only since paper chromatography had allowed to obtain information as to the nature of the hormone analogues and metabolites [3], and the specific metabolic effects and physiological properties of the hormone analogues [4]. While the inhibition of hormone synthesis is directed in the first place against the peroxidase enzyme system, although the enzyme could not be demonstrated in the thyroid proper, recent evidence tends to indicate that in the degradation of thyroid hormones the tryptophan derivatives (serotonin, etc.) play a significant role. It is known from the investigations of Galton and Ingbar [5] that serotonin and the sero-

tonin-liberator reserpine inhibit the metabolization of thyroxine (in the following: T_4) and of triiodothyronine (in the following: T_3) in mammalian and amphibian liver and renal tissue preparations. This inhibition is exerted also when serotonin is not added directly to the tissue preparations, but is administered to the experimental animals (mice, in their case) before study. From the results of the above experiments follows that serotonin inhibits thyroid activity by suppressing the metabolization of thyroid hormones. CERLETTI *et al.* [6] have produced an ergot derivative 1-methyl-d-lysergic acid-butanolamide (Deseril, Sandoz) (in the following, MLAB), which has proved to antagonize serotonin in their experiments. Lysergic acid butanolamide is equally capable of inhibiting the central and peripheral actions of serotonin.

We have assumed that by increasing the metabolization of thyroid hormones MLAB would be capable of stimulating thyroid activity. It might also stimulate thyroid function through the hypothalamic-pituitary system, acting on its central site of action, or even by a direct action on the thyroid. These contentions have been unequivocally corroborated by our results obtained in animal experiments, human patients and normal control subjects.

Materials and methods

Male albino rats (average weight, 193 g) were divided into four groups. They were fed a low-iodine diet [8], containing at the most 0.1 μg of iodine per g. The drugs were administered through a gastric tube and the animals were subjected to study 24, 48, 72 and 168 hours later, respectively. Group 1 (30 rats) served as control, group 2 was treated with 50 mg of methylthiouracil daily, group 3, with 0.25 mg of MLAB daily, while the animals of group 4 with 0.25 mg of MLAB and 50 mg of methylthiouracil daily.

Prior to sacrifice, the animals were given 50 μC carrier-free $K^{131}I$ intraperitoneally (24 hours before subjecting them to study). Then the animals were killed by an overdose of anaesthetic, the thyroid was removed and blood was collected by heart puncture. The material for one experiment was supplied by specimens obtained from 3 animals. The thyroids were weighed by torsion scales and homogenized immediately in 1.5 ml of ice-cool distilled water. From a known volume 0.05 ml samples were applied to two parallel papers and the activity at each point was counted three times by means of a Geiger—Müller counter, in a lead column with the same geometry. The mean impulse/minute values thus obtained were multiplied by 30, expressed in percentage of the amount administered, and computed for 100 mg of tissue this represents the incorporation in the Table [9].

The free hormone content of the thyroid gland was determined radiopaperchromatographically according to TAUROG, TONG and CHAIKOFF [10], after extraction of 1 ml of the homogenate in the presence of a reducing agent with acid butanol, and evaporation. The numerical imp/min value found in the spot T_4 of the paper chromatogram was expressed in percentage of the administered amount and, to facilitate evaluation, was multiplied by 10.000.

The hormone content of blood serum was determined by the same method in the pooled sera from three animals and the blood T_4 values were expressed in the same way.

The inorganic iodine content of blood serum was determined by extraction with chilled butanol, and aliquots of the butanol extract were applied to the paper without loss (without evaporation). The imp/min value of the spot appearing at 0.4 Rf was expressed in per cent of the total radioactive iodine content of the serum.

In vitro experiments

48 hours before killing them, each of 50 rats were injected with 50 μC $K^{131}I$ intraperitoneally, and at 48 hours, when the activity was practically in the T_4 fraction, blood was collected by heart puncture. The blood serum was incubated with Krebs—Ringer phosphate

buffer solution, or with Krebs—Ringer solution + 250 μg serotonin + 125 μg MLAB, at 37° C for 24 hours, then after the butanolic extraction mentioned above the activity found in the single fractions was expressed in percentage of the total activity found in the paper chromatograms, observing the shifts in the ^{131}I values between the single fractions.

Human experiments

Normal volunteers were treated over one week with 1 mg of MLAB t. i. d. The changes in the serum inorganic iodine level were estimated [11], and in a few cases the hormone fractions were separated by paper chromatography and their amounts were determined [12]. Identical examinations have been carried out in patients suffering from hypothyroidism.

Results

I. Incorporation of ^{131}I into the thyroid (Fig. 1)

Group 1. (Control animals).

Incorporation: $\dfrac{\text{mean value} \pm \text{S. D.}}{\text{number of experiments}} = \dfrac{36 \pm 11}{10}$

Group 2. (50 mg MTU daily). The incorporation of ^{131}I was maximally inhibited during the first 24 hours $\left(\dfrac{3.2 \pm 1.2}{3}\right)$, as compared with the imp/min values for the controls. The measure of inhibition did not change substantially in the course of one week $\left(\text{at 168 hours} = \dfrac{5.1 \pm 1}{5}\right)$.

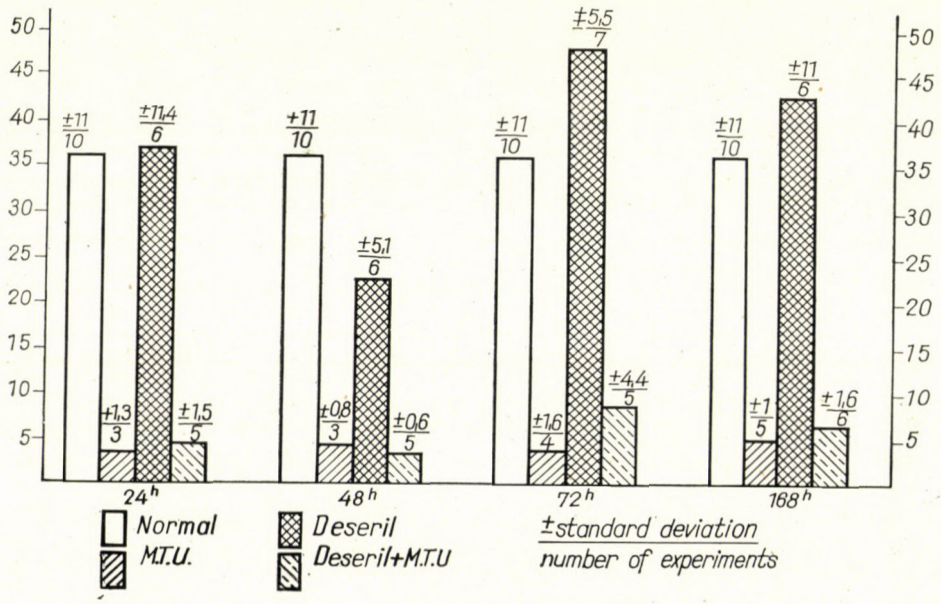


Fig. 1. ^{131}I uptake by the thyroid, in percentage of the dose administered

Group 3. (0.25 mg MLAB daily). During the first 24 hours ^{131}I incorporation did not differ considerably from that of the controls: $\frac{36.8 \pm 11.4}{6}$. At 48 hours a slight decrease was noted: $\frac{22 \pm 5}{6}$. A significant increase followed after 72 hours: $\frac{48.5 \pm 5.5}{7}$, and the higher incorporation values persisted until the end of the one week period of observation: $\frac{43.4 \pm 11}{6}$.

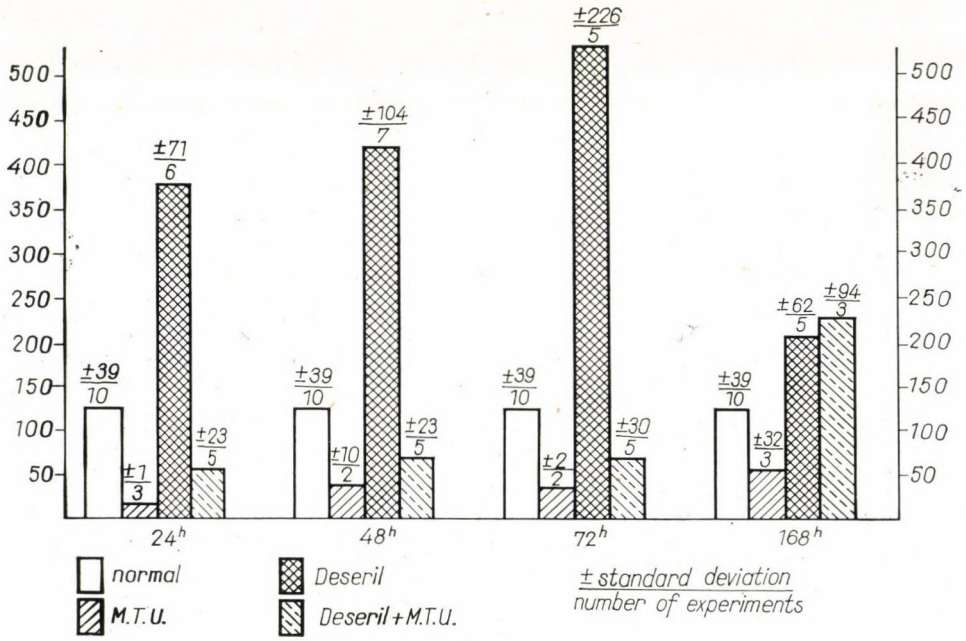


Fig. 2. Free $T_4{}^{131}I$ in thyroid, in percentage of ^{131}I injected. $\times 10^4$

Group 4. (0.25 mg MLAB + 50 mg MTU daily).

The 24-hour iodine uptake by the thyroid was much lower than that in the controls $\left(\frac{3.8 \pm 1.5}{10}\right)$, but no such maximal suppression resulted as in the MTU-treated animals. At 48 hours the incorporation value was $\frac{3.14 \pm 0.6}{11}$. The 72-hour $\left(\frac{8.3 \pm 4.4}{9}\right)$ and 168-hour values $\left(\frac{6.86 \pm 1.6}{6}\right)$ definitely exceeded the iodine uptake by the thyroid observed in MTU-treated animals.

II. Free $T_4^{131}I$ in the thyroid, in percentage of the amount of ^{131}I injected (Fig. 2)

Group 1. (Controls).

Free $T_4^{131}I$ in thyroid $= \frac{126 \pm 39}{8}$.

Group 2. (50 mg MTU).

At 24 hours there was hardly any free $T_4\,^{131}I$ in the thyroid $\left(\frac{16 \pm 1}{2}\right)$. There was a slight increase at 48 and 168 hours, the values being $\frac{39 \pm 10}{2}$, then $\frac{35 \pm 2}{2}$, and finally $\frac{55 \pm 32}{3}$.

Group 3. (0.25 mg MLAB daily).

The $T_4\,^{131}I$ content of the thyroid greatly increased during the first 24 hours $\frac{377 \pm 71}{6}$, to reach $\frac{417 \pm 104}{7}$ at 48 hours, and the maximum of $\frac{531 \pm 226}{5}$ at 72 hours, a value many times the control one. The free $T_4\,^{131}I$ content of the thyroid was still very high by the end of the experiment, at 168 hours $\left(\frac{207 \pm 62}{5}\right)$. The measure of the increase of T_4 content was much greater than the increase in the rate of incorporation, *i. e.* more inorganic ^{131}I was converted to $T_4\,^{131}I$ in the unit of time (increased conversion rate).

Group 4. (0.25 mg MLAB + 50 mg MTU daily).

At 24 hours the T_4 content of the thyroid was about half the normal $\left(\frac{55 \pm 23}{10}\right)$. Later, it increases gradually and by 168 hours it is considerably higher than the values for the normal controls and even more above that of the MTU-treated group $\left(\frac{224 \pm 94}{6}\right)$.

III. $T_4\,^{131}I$ in serum, in percentage of the amount of ^{131}I injected $\times 10^4$ (Fig. 3)

Group 1. (control animals). The $T_4\,^{131}I$ content of serum $= \frac{26 \pm 17}{8}$.

Group 2. (50 mg MTU daily). The sera from the animals killed at the specified points of time showed about the same $T_4\,^{131}I$ values, reflecting the condition corresponding to maximal inhibition. The values were at 24 hours $\frac{1 \pm 0}{3}$, at 48 hours $\frac{2 \pm 0.7}{3}$, at 72 hours $\frac{3 \pm 1.7}{4}$, and at 168 hours $\frac{20 \pm 4}{3}$.

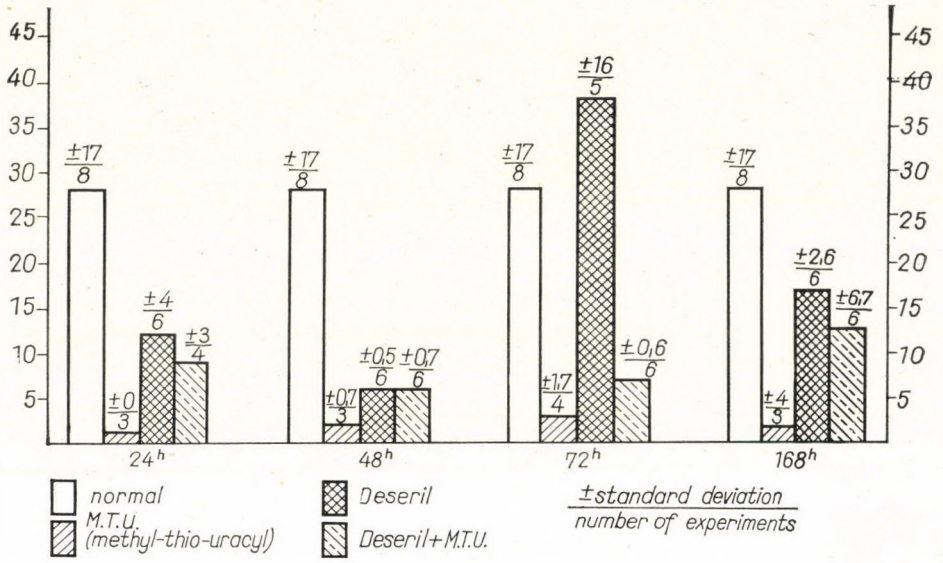


Fig. 3. T_4 ^{131}I in serum, in percentage of ^{131}I administered, multiplied by 10^4

Group 3. (0.25 mg MLAB daily).

The 24-hour value $\frac{12 \pm 4}{6}$, about half the value found in the untreated controls. The 48-hour value was still lower $\left(\frac{6 \pm 2}{7}\right)$, while the 72-hour one much higher than the normal control value $\left(\frac{38 \pm 16}{5}\right)$. The value found at 168 hours was $\frac{17 \pm 2}{6}$.

Group 4. (0.25 mg MLAB + 50 mg MTU daily).

The serum T_4 ^{131}I values in this group were at 24 hours, $\frac{9 \pm 2.6}{8}$; 48 hours $\frac{6 \pm 0.7}{12}$. From the 72nd hour on the values tended to increase, reaching $\frac{13 \pm 6.7}{6}$ at 168 hours, a peak five times higher than the value for the rats treated with MTU alone, but still below the control level.

IV. Changes in thyroid weight, computed for 100 g of body weight (Fig. 4)

Group 1. (control animals). The average gland weight per 100 g body weight was 9.8 mg.

Group 2. (50 mg MTU daily). The average thyroid weight of 9.8 mg increased to almost 16 mg by 24 hours. The 48-hour value was 15 mg, the 72-hour one was 12.0 mg. The 168-hour value was 19.0 mg.

Group 3. (0.25 mg MLAB daily). It was remarkable that the values for the first three days were almost identical with those found for the animals treated with the thyroid-inhibitor MTU. During the first 24 hours the value increased steeply to 13 mg, and continued to increase on the second day to 15.0 mg. On the third day thyroid weight dropped to a value lower than the

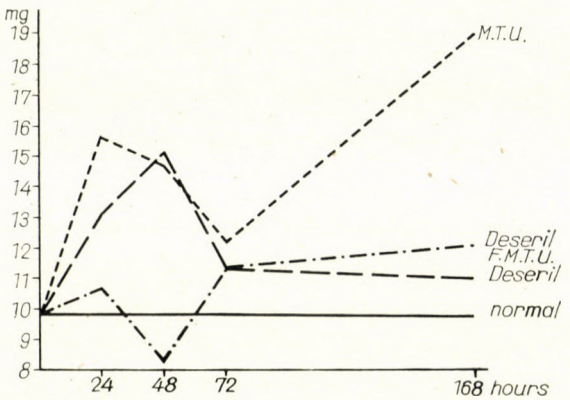


Fig. 4. Changes in thyroid weight, computed for 100 g body weight

24-hour one (11.58 mg). Subsequently thyroid weight persisted at a similarly low level (11.0 mg).

Group 4. (0.25 mg MLAB + 50 mg MTU daily).

At 24 hours the thyroid weighed 10.4 mg. Like in group 2, the weight decreased by the 48th hour (8.2 mg). The 72 hour value was 11.2 mg, to remain practically unchanged (12.0 mg) until the end of the experiment.

V. Serum inorganic ^{131}I in percentage of serum activity, during treatment with 0.25 mg of MLAB daily (Fig. 5)

At 24 hours the inorganic ^{131}I value was 36.3 per cent, practically identical with that for the untreated controls (37 per cent). At 48 hours the value was significantly increased (46.6 per cent), at 72 hours again less (27.3 per cent), to rise subsequently to 50.3 per cent at 168 hours.

VI. Experiments with rat sera incubated with serotonin, or with serotonin and MLAB (Table 1)

In the basic experiment, rat blood serum obtained 48 hours after a dose of ^{131}I was incubated in Krebs—Ringer phosphate buffer for 24 hours. The activity values were 18.5 per cent for inorganic iodide, 46.8 per cent for T_4,

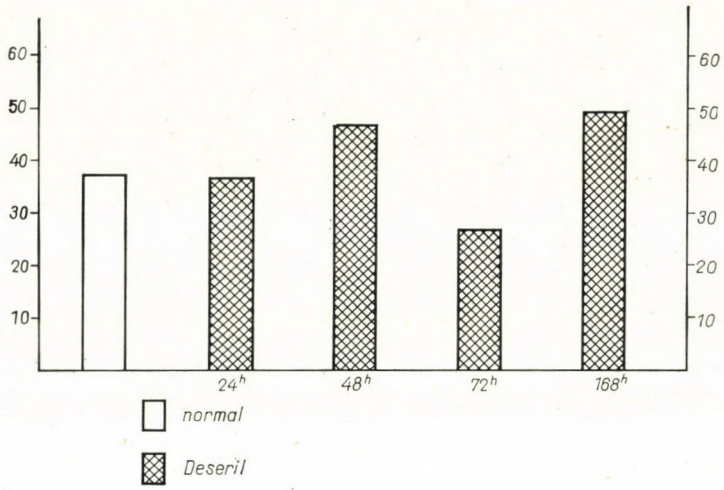


Fig. 5. Inorganic ^{131}I in serum, in percentage of serum activity

Table 1

Paper chromatographic distribution of ^{131}I, in percentage of the total activity applied
(Mean values)

	Number of experiments	Site of activity (Rf values)					
		start	0.11 DIT	0.36 iodide	0.4 T_4	0.6 T_3	front
1. Rat serum, duration of incubation 48 hrs. Extracted with butanol	6	3.7	3.5	8.1	69.9	12.4	2.6
2. Incubation in Krebs—Ringer phosphate buffer	6	7.2	8.4	18.5	46.8	15.3	7.7
3. The former + 250 μg serotonin...	5	4.6	6.6	8.4	65.4	11.2	3.3
4. Incubation in Krebs—Ringer phosphate buffer + 250 μg serotonin + 125 μg MLAB	6	8.8	10.9	20.7	38.8	14.4	4.5

Incubation lasted 24 hours in a 37° C water bath in every case.

and 15.3 per cent for T_3. After incubation with 250 μg of serotonin the inorganic iodine value decreased to 8.4 per cent, that for T_4 was 65.4 per cent and the T_3 value, 11.2 per cent. There was a conspicuous change in the T_4/T_3 ratio (control group: 3 : 1, serotonin group 5.9 : 1). The values for group 3 (250 μg of serotonin + 125 g of MLAB) were: inorganic iodide 20.7 per cent, T_4 38.8 per cent and T_4 14.4 per cent. In this group the value of the T_4/T_4 ratio was 2.6 : 1.

VII. Histological studies

At 24 hours following the administration of MLAB the rat thyroid showed the following histological pattern (Micrographs 1 to 3). The acini are different in size, the acinar epithelium is flattened, cuboid-shaped, the enlarged glands are filled by homogeneous colloid, a picture characteristic of normal function. After 2 days of treatment the lumina of the glands are narrow, the follicular epithelial elements lining the lumina are higher, the acini are filled by detached epithelial cells, thus the picture points to acute hyperfunction. At 72 hours acini of narrow lumen lined by high cuboid epithelium are found in the richly glandular thyroid tissue, with occasional groups of lymphocytes in the interstitium. At 168 hours dilute colloid fills the slightly dilated acini, and interstitial, septal lymphoid and connective tissue proliferation is visible. The histological pattern resembles that of human hyperfunctioning goitre.

VIII. Human experiments

Group 1. Each of the 5 healthy control subjects was treated with 1 mg of MLAB t. i. d. for one week. Prior to treatment, each one of them was tested for serum inorganic iodine with the following results:

K. J.: 3.0; Gy. E.: 3.1; G. F.: 3.8; Mrs. L. Sz.: 4.0; G. T.: 4.5 μg per 100 ml.

The tests made after the one week of treatment yielded the following results:

K. J.: 4.4; Gy. E.: 4.4; G. F.: 4.9; Mrs. L. Sz.: 4.9; G. T. 5.3 μg per 100 ml.

The average increase was 1.1 μg per cent. The highest rise was found in the case of K. J., who had shown one of the lowest initial serum iodine values, and the smallest was found in the case of G. T., who had shown the highest organic iodine level prior to treatment.

Group 2. Seven patients suffering from myxoedema or latent hypothyroidism were treated with 1 mg of MLAB t. i. d. for one week. Before treatment, the serum organic iodine values were:

Mrs. F. I.: 1.5; Mrs. K. J.: 2.7; Mrs. B. I.: 3.2; Mrs. H. M.: 3.6; Mrs. K. I.: 4.1; Mrs. N. E.: 5.6; Mrs. Sz. I. 3.8 μg per 100 ml.

After treatment the values were:

Mrs. F. I.: 3.3; Mrs. K. J.: 4.7; Mrs. B. I.: 5.0; Mrs. H. M.: 4.1; Mrs. K. I.: 6.4; Mrs. Sz. I.: 5.0; Mrs. N. E.: 6.0 μg per 100 ml.

The average increase was 1.68 μg per cent. Here, too, the increment was largest with the patient showing the lowest initial value, and smallest in the case of N. E., whose organic iodine level in serum had been normal. It is to be noted that, as determined by the method used at our Institute, the normal serum organic iodine values range from 3.8 to 7.5 μg per 100 ml.

Discussion

In our experiments and clinical studies the compound lysergic acid butanolamide, which has antiserotonin activity, has proved unequivocally to increase thyroid activity.

Except for the 48-hour values, in the animal experiments the incorporation of ^{131}I was above the normal value. After the first day the free T_4 content

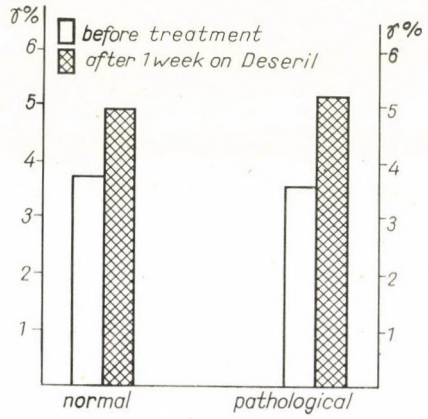


Fig. 6. Serum organic iodine, mean, μg per cent

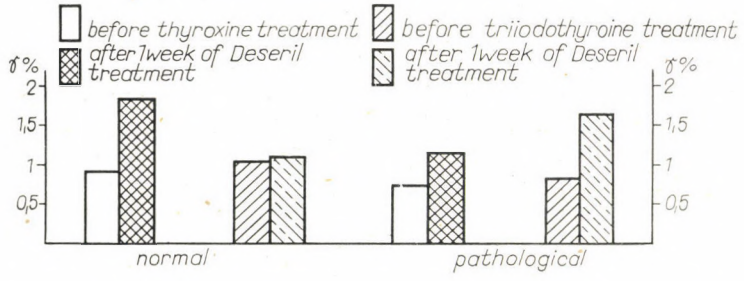
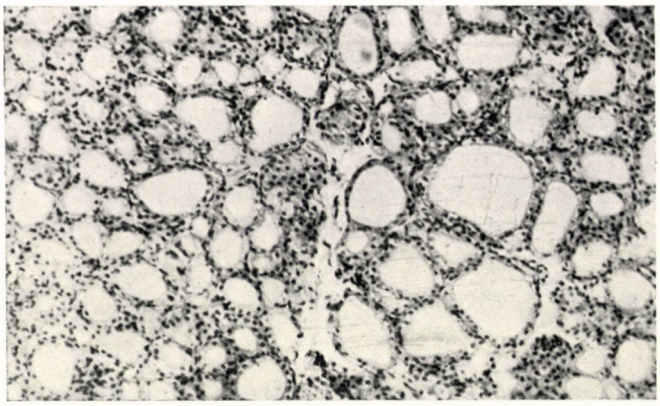
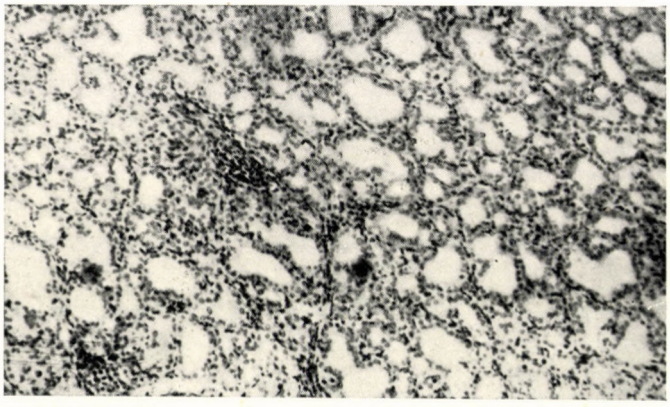


Fig. 7. Serum thyroxine and triiodothyronine, in μg per cent

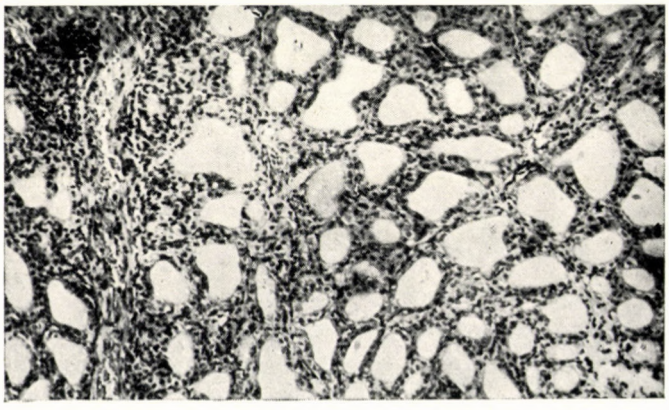
of the thyroid was strongly increased and continued to increase gradually till 72 hours. However, incorporation and free T_4 content did not increase at the same rate; the latter increased much faster (increased conversion rate; faster utilization of the incorporated iodine). In addition, an immediate, quick breakdown of thyroglobulin may play a role in the increase of the free T_4 content of the thyroid, but our method did not lend itself to an establishing the rate of that process. The increase of the free T_4 content of the thyroid seems to be a result of TSH action. Incorporation, glandular and serum T_4 contents reached



Microphotogram 1. 24 hours after MLAB treatment



Microphotogram 2. 48 hours after MLAB treatment



Microphotogram 3. 72 hours after MLAB treatment

the maximum at 72 hours. At that time the T_4 content of serum was above normal — while otherwise always below normal —, which may be explained by an increase of deiodination caused by MLAB. The maximum T_4 output at 72 hours has been confirmed also by the change in thyroid weight, which reached the minimum at 72 hours. The deiodination-increasing effect of MLAB has been proved also by the inorganic ^{131}-iodine contents of the sera tested; except for the 72-hour values these were always over the normal level.

The results of our *in vitro* experiments, too, indicated that the degradation of the iodine hormones is increased by MLAB. In the rat sera incubated for 24 hours with serotonin the amount of the deiodination products of iodine hormones: inorganic iodine, T_3 and diiodotyrosine, decreased as a result of the inhibition. If MLAB had been added to the mixture, the amount of each of the three fractions mentioned above increased, and even surpassed the values found in control serum. The simultaneous administration of MTU and MLAB supplied further information as to the stimulation of thyroid function by serotonin antagonists. As compared with the animals treated with MTU alone, the uptake of labelled iodine hardly increased, but the free T_4 content of the thyroid increased almost fivefold. The serum T_4 level increased approximately at the same rate. This result may be interpreted as indicating that MLAB prevents MTU from exerting its full inhibitory effect on the synthesis of the thyroid hormones (peroxidase system). Furthermore, our results may be explained by the increase of the conversion rate caused by MLAB (see group 1.). The MLAB—MTU antagonism is reflected also by the weight curves. When the two drugs are administered simultaneously it is namely found that the weight of the thyroid does not increase fivefold, because MLAB, although it causes some increase of thyroid weight itself, counteracts the goitrogenic effect of MTU.

The excessive increase of thyroid weight observed 72 hours after the administration of MTU, and attributed to the effect of TSH, does not take place when MLAB is applied in combination with MTU; in this case the shape of the weight curve was almost identical with that of the control animals. In other words, MLAB is capable of compensating the effect of inhibited uptake, thus satisfactorily ensuring peripheral requirement (the metabolisation of thyroid hormones increases). This is why no substantial TSH effect is observed.

There is no divergence between the histological pattern of the thyroid and the functional data in the case of the MLAB-treated animals. On the second day already the picture suggests acute hyperfunction. Later, alongside the hyperactive epithelial elements lymphoid and connective tissue infiltrations also appear, indicative of a maximal glandular hyperfunction, when the active epithelial elements are no longer capable of further increasing production and, as a result of the relative exhaustion, lymphoid and connective tissue propagation develops.

The results of our human observations agreed with those of the animal experiments. After one week of treatment the initial serum organic iodine level was increased by an average of 1.3 μg per 100 ml in healthy volunteers. They gradually developed characteristic clinical symptoms, such as episodes of excitement, slight sweating, mild vertigo, palpitation, insomnia and loss of concentrative power. No toxic side-effect making necessary to discontinue the treatment has been observed. Then patients suffering from manifest or latent myxoedema were treated with the drug, likewise for one week. The serum organic iodine level increased more markedly then in the controls, by an average of 1.6 μg per 100 ml. In three of these cases chromatographic analysis for thyroid hormones was also carried out. The serum T_4 fraction was increased both in the patients and in the normal subjects; in the latter to nearly double the initial. After one week of treatment the amount of T_3 slightly decreased in the normal subjects, while it markedly increased in the patients, reaching double of the initial value after one week of treatment. This phenomenon might find its explanation in an increased degradation of thyroid hormones.

The subjective complaints soon subsided in the MLAB-treated myxoedematous patients, with a marked improvement of somnolence, fatigue and eventually of depression. These patients, unlike the normal test subjects, complained of no untoward side effects.

The results obtained tend to prove that MLAB increased thyroid function in every one of the parameters examined by us. The underlying mode of action is unclear. An enhanced degradation of thyroid hormones should be taken into consideration in the first place, in view of the drug's antiserotonin activity. The results obtained suggest that MLAB does in fact increase hormone degradation, because in the serum of treated animals the inorganic iodine labelled with ^{131}I showed a higher level than in that of the controls.

The increased metabolization of thyroid hormones by itself does not fully explain the sudden increase of thyroid function, which in the animals manifested itself in an increase of free thyroid T_4 content after the first 24 hours already, and in every aspect of thyroid hyperfunction after two days. The increase of the serum organic iodine level by more than 1 μg per 100 ml noted in the patients after one week of treatment was another change that may be interpreted as being indicative of a significant increase in thyroid function.

A direct stimulating effect might also be involved, according to SAUNDERS [13], in the brain and liver, through the inhibition of monoamino-oxidase, the serotonin level increases, causing a reduction of the diphosphopyridine nucleotide level. Through a similar mechanism serotonin may increase in the thyroid, too, which contains serotonin in substantial amounts. The thyroid gland develops from the digestive tract and thus possesses cells of the enterochromaffine type [14, 15, 16]. Serotonin may not only inhibit the degradation of thyroid

hormones, but also decreases the thyroid DPN level. On the other hand, DPN increases in the thyroid the iodination of tyrosine [7], directly and indirectly (the activity of the HMP shunt increases as a result of the increased TPNH synthesis). It may therefore be assumed that in DPN deficiency the thyroid hormones are synthesized at a reduced rate, and at an increased rate when the level of DPN is increased as a result of serotonin inhibition [16].

Moreover, MLAB increased the activity of TSH. The prompt increase of thyroid weight in response to MLAB may have been the result of a TSH stimulus. In other words, the stimulation of pituitary activity is realized by central action, through the diencephalic site of action of MLAB.

A further finding was the sudden increase of iodine incorporation on the third day and the simultaneous maximum thyroid hormone output (the high serum T_4 value at 72 hours, and a decrease of thyroid weight occurring at the same time). These appear to be due to TSH action. According to MITCHELL [19], the inhibitory effect of thiouracil is diminished by TSH. Our results indicate that MLAB diminishes MTU inhibition, presumably through an increase of TSH secretion. Further investigations are planned to study the effect of MLAB on TSH secretion.

Lysergic acid butanolamide, which stimulates equally the thyroid hormone synthesizing and metabolizing activities of the thyroid, seems to be suitable for induction of experimental hyperthyroidism, and thus for studying the pathophysiology of hyperthyroidisms.

Acknowledgements

We are indebted to Dr. D. TANKA for the histological examinations, and to the Scientific Department of the firm *Sandoz* (Basel) for supplies of the experimental material.

REFERENCES

1. ASTWOOD, E. B.: J. Pharmacol. exp. Ther. **78,** 79 (1943).
2. MACKENZIE, C. G., MACKENZIE, J. B.: Endocrinology **32,** 185 (1943).
3. STANBURY, J. B.: Ann. Acad. Sci. **86,** 417 (1960).
4. ROCHE, J., MICHEL, R.: Physiol. Rev. **35,** 583 (1955).
5. GALTON, W. A., INGBAR, S. H.: Endocrinology **69,** 834 (1961).
6. CERLETTI, A., DOEPFNER, W.: Helv. physiol. pharmacol. Acta **16,** 55 (1958).
7. FANCHAMPS, A., DOEPFNER, W., WEIDMAN, H., CERLETTI, A.: Schweiz. med. Wschr. **90,** 1040 (1960).
8. TAUROG, A., CHAIKOFF, I. L.: J. biol. Chem. **165,** 217 (1946).
9. SZÁNTÓ, L., L. REVICZKY, A.: Arzneimittel-Forsch. **13,** 214 (1963).
10. TAUROG, A., TONG, W., CHAIKOFF, I. L.: J. biol. Chem. **184,** 83 (1950).
11. BARKER, S. B.: J. clin. Endocr. **10,** 1136 (1950).
12. R. BALOGH, I.: Orv. Hetil. **41,** 1136 (1950).
13. SAUNDERS, J. C.: Psychosomatics **1,** 86 (1960).
14. PAASONEN, M. K.: Experientia (Basel) **14,** 95 (1958).

15. Eggerth, B.: Morphologie und Histophysiologie der normalen Schilddrüse. p. 27. Leipzig, 1938.
16. Bremer, J. L., Weatherford, M. L.: A Textbook of Histology VI. pp. 440—443. Philadelphia, 1944.
17. Klebanoff, S. J., Yip, C.: Fed. Proc. **20**, I. 202 (1961).
18. Schussler, G. C., Ingbar, S. H.: J. clin. Invest. **40**, 1394 (1961).
19. Mitchell, M. L.: J. clin. Endocr. **21**, 1566 (1961).

Dr. László Szántó, Budapest II. Frankel Leó út 17/19, Hungary
Dr. Alice L. Reviczky, Tamás Grynaeus } Budapest II. Frankel Leo út 25/27, Hungary

CORRELATION BETWEEN CAPILLARY FILTRATION AND LYMPH FLOW IN VENOUS CONGESTION

By

GY. SZABÓ, ZSUZSA MAGYAR and M. PAPP

FIRST DEPARTMENT OF MEDICINE, UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received May 31, 1962)

The effect of venous congestion in the hind legs of anaesthesized dogs on the fluid and protein loss from the capillaries, on the quantity and protein content of the lymph flowing from a lymphatic of the extremity has been investigated. Between fluid filtration and lymph flow a significant, positive, though not very close correlation ($r = 0.29$ $p < 0.05$) has been found. Lymph flow increased parallel with the increase of venous pressure according to the regression equation, $L = 76.3 + P \cdot 4.01$. The protein content of the lymph was much higher than the calculated protein concentration of the capillary filtrate. No correlation could, however, be found between the two values ($r = 0.02$).

The rate of flow of fluid filtering through the capillary wall under constant colloid-osmotic and tissue pressures is directly proportional to the capillary pressure. The increase of venous pressure reacting upon the capillary pressure accordingly enhances capillary filtration [1, 2]. With the increase of capillary filtration the flow in the lymph vessels of the respective area usually also increases. Thus, due to the venous congestion brought about in a limb, lymph flow increases in the foot [3, 4].

WHITE, FIELD and DRINKER [5] exerting pressure on the hind legs of dogs observed an increase of lymph flow. There having been no proper investigations of quantitative character, it has been impossible to perform an exact analysis of the correlation between the rise of venous pressure and the increase of capillary filtration and the changes of the lymph flow, respectively.

Methods

The investigations were performed in 18 dogs weighing 15—20 kg, anaesthesized with hexobarbital-sodium. The femoral artery and vein were prepared under the inguinal region on one leg and a close tourniquet led under the lifted vessels was applied on the limb. A rotameter and a manometer for measuring blood flow and pressure were tied into the artery. A polythene cannula was introduced into the femoral vein through a distal lateral branch, and was connected with a water manometer. Then the popliteal lymph node was stitched round with thread and tied (Fig. 1). From among the dilated lymph vessels a suitable one was chosen and a cannula was tied into it to collect lymph. The limb was passively moved at a frequency of 30 per minute, as described elsewhere [6].

After some periods of collecting lymph lasting generally 10 minutes, venous pressure in the limb was increased by narrowing the femoral vein. The pressure values could be regulated by changing the rate of narrowing. In the control periods as well as at higher pressure values blood samples were repeatedly withdrawn from the femoral artery and vein, haematocrit and

protein concentration were determined. On the basis of these results and the blood flow measured with the rotameter the quantity of fluid filtrating in the leg per minute as well as the protein content of the filtrate were calculated.

Fig. 1. Popliteal lymph nodes and lymph vessels in the hind leg of the dog
1 — place of ligating the lymph node;
2 — cannula in a lymph vessel

Results

The average quantity of lymph flowing from the opened lymph vessel amounted to 19 $\pm$ 17 mg per minute. The flow of lymph as a rule increased

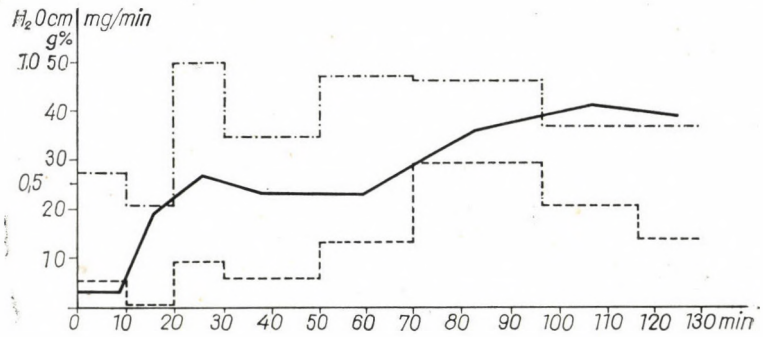


Fig. 2. Lymph flow and capillary filtration in venous congestion
——————— venous pressure (cm H_2O)
— — — — — flow of lymph (mg/min)
—.—.—.— protein content of lymph (g per cent)

with the rise of venous pressure. But this increase was by no means as regular and immediate as stated by WHITE *et al.* [5], it sometimes occurred with a delay of 10—20 minutes and often decreased on prolonged venous congestion (Fig. 2). On the other hand, the flow of lymph did not immediately return to the starting value when congestion had ceased. Considering the fact that lymph flow was different from animal to animal even before constricting the femoral vein (at a venous pressure of 2.5 to 7.5 cm H_2O), the flow observed before the periods of venous congestion was regarded as the basic value (100 per cent) and to this were compared the changes of lymph flow (Table 1). All the data

of 102 observations are shown in Fig. 3. In spite of the wide scattering, lymph flow showed a definite increasing tendency when venous pressure increased, and there proved to be a lineal connection between the values of pressure and flow, expressed by the regression equation:

Table 1

Increase of the lymph flow and rise of venous pressure

P	0–10	10–20	20–30	30–40	40–50	50–60	Above 60
L%	100.0	122.2	151.5	315.2	195.7	318.0	320.2
S D.		86.0	77.5	232.6	87.9	223.7	284.5
n.	—	31	17	18	12	6	18

P = venous pressure cm H_2O;
L% = average lymph flow expressed in percentage of flow in control-periods;
S. D. = standard error;
n. = number of observations.

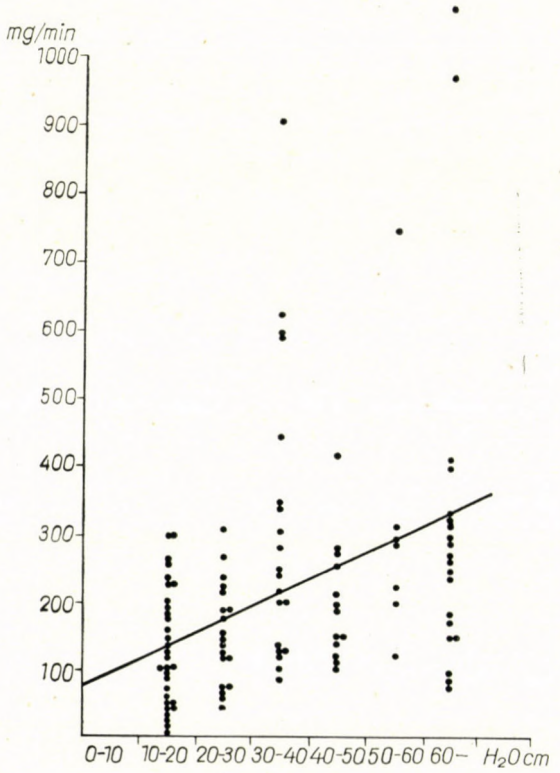


Fig. 3. Correlation between lymph flow (mg/min) and venous pressure (cm H_2O)

$$L = 76.3 + 4.01\ P$$

where L means the flow of lymph (mg/min) and P the venous pressure (cm H_2O). According to the equation a 40.1 per cent increase of lymph flow corresponds to every 10 cm rise of venous pressure.

The rise of venous pressure increased the capillary filtration in the limb. The loss of the filtered fluid amounted to an average of 0.186/min in the periods preceding the venous congestion (Table 2). The number of observations, however, did not suffice for a mathematical analysis of the results as was done with the lymph flow values.

Table 2

Effect of venous congestion on the amount of filtrated fluid and the rate of lymph flow

P	0–10	10–20	20–60	Above 60	Total
FR ml/min	0.186	0.964	0.980	3.204	—
S. D.	0.40	2.85	8.24	2.77	—
L ml/min	0.019	0.020	0.026	0.053	0.027
S. D.	0.017	0.015	0.010	0.026	0.011
n	17	14	16	8	53

A significant increase of filtration was observed to occur only when pressure increased above 60 cm H_2O. This increase was analyzed by the χ^2 method; this revealed that increased filtration values were significantly ($p < 0.02$) at a pressure above 60 cm H_2O more frequent than at lower venous pressure.

The relation of the filtration values to the flow of lymph observed at the same points of time was also examined (Fig. 4). The correlation coefficient ($r = 0.29$) showed that between the two values there exists a limited, but significant ($p < 0.05$) positive correlation.

The protein content of the filtrate was 0.79 g per 100 ml in the periods before venous congestion. With the increase of venous pressure it showed a definite rising tendency (Table 3). The rise was, however, significant only at pressure values above 60 cm H_2O (Student's "t" method yielded $t = 2.581$, $p < 0.02$). Between the pressure values of 20 and 60 cm H_2O, the increase did not prove to be significant ($t = 1.087$, $p > 0.20$). The lack of significance is in all probability explained by the unsatisfactory number of observations. The protein content of the lymph, too, showed a minimal rise during venous congestion. No definite correlation could be shown between the protein content of the filtrate and the protein concentration of the lymph collected simultaneously ($r = 0.02$).

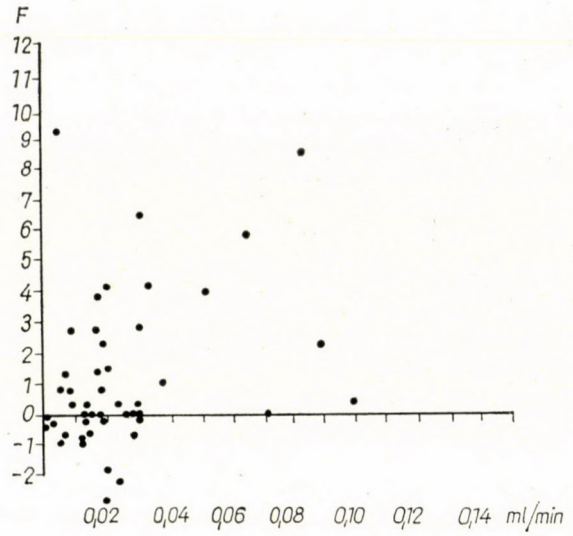


Fig. 4. **Correlation between capillary filtration and lymph flow (mg/min)**

Table 3

Protein content of capillary filtrate and lymph

P	0–10	10–20	20–60	Above 60	Total
Fp	0.79	0.79	2.06	3.20	1.46
S. D.	2.87	3.59	3.37	2.02	3.26
Lp	2.00	2.04	2.38	2.39	2.15
S. D.	0.15	0.26	0.21	0.86	1.02
n	15	11	11	8	45

P = venous pressure, cm H_2O;
Fp = percentage of protein content of the filtrate, on the basis of the formula,

$$Fp = \frac{Pa\,(100 - Ha) - Pv\,(100 - Ha - F)}{F}$$

where *Pa* and *Pv* mean the protein content of arterial or venous blood, *Ha*, the arterial haematocrit, and *F*, the quantity of capillary filtrate.
Lp = protein content of lymph in g per 100 ml.

Discussion

A positive correlation has been shown to exist between the increased filtration of fluid in the limb and the rate of lymph flow. This correlation, however, is not so close as one might expect on the basis of earlier data in the literature. The probable reason for this is that the flow of lymph is dependent

on the quantity of extracellular fluid in the tissue and the tissue pressure rather than on momentary capillary filtration. The increased filtration first fills up the extracellular space and only then will start the increased lymph flow. The increase of lymph flow does not necessarily coincide in time with the increase of filtration; it starts later and lasts longer. The change of lymph flow may, on the other hand, depend on the original water content of the tissues. Our present experiments, however, do not offer sufficient proof of this supposition. It must be pointed out however that in our experiments the value for capillary filtration shows the state at the moment withdrawal of the blood sample, while lymph collection takes 10 to 20 minutes, thus lymph flow values represent only a mean for the given period.

We have, furthermore, stated that between venous pressure and increase of lymph flow there is a correlation of the same quantitative character as between the rise of venous pressure and the increase of capillary filtration [2, 7, 8]. The correlation shown by us is valid for venous pressures between 10 and 80 cm H_2O. The "critical pressure value" described in the human limb by KROGH, LANDIS and GIBBON [1] stating that filtration increases only at venous pressure values above 15 cm H_2O has not been observed by us in connection with lymph flow, nor has in connection with capillary filtration in the cat limb such a critical pressure been observed by PAPPENHEIMER and SOTO-RIVERA [8].

The rate of capillary filtration computed from the haematocrit value was at least ten times as much as the quantity of the lymph flowing from the opened lymph vessel. Disregarding some possible methodical errors, we have to point out that only one lymph vessel was cannulated by which vessel the lymph is supplied only from the lower leg and not from the thigh. On the other hand there are at least 3—4 other lymph vessels of similar size removing the lymph of this area. It must, however, be pointed out that all lymph vessels had been obstructed, thus flow in the opened lymph vessels was in all probability greater than under normal circumstances.

The mean protein content of the capillary filtrate was 0.79 g per 100 ml, higher than the mean of 0.3 g per 100 ml found by LANDIS *et al.* [9] in the human limb. From this difference no far-reaching consequences can be drawn, as it may have been due to differences either in species or methodology. This value, however, is definitely lower than the protein content of the lymph simultaneously obtained. We cannot regard this fact as being only natural. According to LANDIS *et al.* as well as our own investigations, the filtration of fluid and protein represents a net loss, i. e. the difference of capillary filtration and absorption. It was therefore striking that the protein content of the lymph carrying off the difference of filtration and absorption was not the same as that of the fluid lost from the blood stream. It necessarily follows that in the area in question a fluid free from, or very poor in, protein was being accumulated. Raising this question is also justified by one of our observations showing

a decrease of the protein concentration of peripheral lymph in chronic venous congestion (10); the protein content approached that of the filtrate under continuously increased filtration rate in spite of the increased filtration of protein. Acute experiments have not revealed anything similar. It is obvious that some time is needed to reach a dynamic state between the respective protein concentrations of the filtrate, the extracellular fluid and the lymph and it is easy to understand why we have failed to find a correlation between the protein content of the lymph and the filtrate.

REFERENCES

1. KROGH, A., LANDIS, E. M., TURNER, A. H.: J. clin. Invest. **11,** 63 (1932).
2. PAPPENHEIMER, J. R.: Physiol. Rev. **33,** 387 (1897).
3. SOTNISCHEWSKY: Virchows Arch. path. Anat. **77,** 85 (1879).
4. HAYNES, F. W.: Amer. J. Physiol. **101,** 223 (1932).
5. WHITE, J. C., FIELD, M. E., DRINKER, C. K.: Amer. J. Physiol. **103,** 34 (1933).
6. SZABÓ, GY., MAGYAR, ZS.: Acta med. Acad. Sci. hung. **8,** 287 (1955).
7. LANDIS, E. M., GIBBON, J. H.: J. clin. Invest. **12,** 105 (1933).
8. PAPPENHEIMER, J. R., SOTO-RIVERA, A.: Amer. J. Physiol. **152,** 471 (1948).
9. LANDIS, E. M., JONAS, L., ANGEVINE, M., ERB, W.: J. clin. Invest. **11,** 717 (1932).
10. SZABÓ, GY., MAGYAR, ZS., ZOLTÁN, Ö. T.: In press.

Dr. György SZABÓ, National Institute of Traumatology, Budapest VIII. Mező Imre út 17, Hungary

Dr. Zsuzsa MAGYAR
Dr. Miklós PAPP
} Budapest VIII. Korányi Sándor u. 2/a, Hungary

Printed in Hungary

A kiadásért felel az Akadémiai Kiadó igazgatója — Műszaki szerkesztő: Farkas Sándor
A kézirat nyomdába érkezett: 1963. III. 13. — Terjedelem: 8,75 (A/5) ív, 36 ábra

63.56831. Budapest, Akadémiai Nyomda — Felelős vezető: Bernát György

ACTA MEDICA

ТОМ XIX — ВЫП. 2

РЕЗЮМЕ

ИССЛЕДОВАНИЕ МЕХАНИЗМА ОБРАТНОГО РАЗВИТИЯ НЕСАХАРНОГО ДИАБЕТА У КРЫС ПОСЛЕ РАЗРУШЕНИЯ НОЖКИ ГИПОФИЗА

К. КОВАЧ, М. А. ДАВИД и Ф. А. ЛАСЛО

Авторы при помощи аппарата Хорслей—Кларка повреждали ножку гипофиза у крыс, и спустя несколько недель после вмешательства исследовали водный режим животных.

Было установлено, что в случае пероральной нагрузки водопроводной водой полиурическая реакция, наблюдаемая после вмешательства, у большинства животных постепенно прекращается и 1—2 месяца после операции количество мочи животных уже не превышало или только весьма мало превышало средние величины у контрольных крыс. После дачи этанола, при введении небольшого количества физиологического раствора или кортизона, удалось вызвать значительную полиурию. Экзогенный креатининовый клиренс снижался и удельный вес как и содержание электролитов мочи были более низкие. Прекращение состояния, напоминающего несахарный диабет, однако, только кажущееся, так как самопроизвольный прием воды у животных даже спустя несколько недель после операции еще оставался повышенным. Отмечается, что противодиуретическая активность в гипоталамусе значительно снижена, а в неврогипофизе ее почти нельзя выявить. Морфологически имеется значительная атрофия и исчезновение невросекреции.

Результаты указывают на то, что производство противодиуретического гормона спустя несколько недель после вмешательства не восстанавливается, и следовательно, этот гормон не играет роли при обратном развитии полиурической реакции. Прекращение повышенного диуреза можно отнести прежде всего к снижению клубочковой фильтрации, но возможно, что в этом механизме участвуют также другие факторы. Изменение почечного кровообращения предположительно обуславливается отчасти гипофункцией оси гипофиз-кора надпочечников, развивающейся вследствие повреждения ножки гипофиза.

О ЗАПАЗДЫВАНИИ ОБЪЕМНЫХ ВОЛН ВЕННОЙ КРИВОЙ

ДЬ. БОДРОГИ и А. КОВАЧ

Венная кривая состоит в основном из объемных волн. Для распространения волн необходимо определенное время, так как скорость распространения весьма незначительна. На более отдаленных от сердца венах волны появляются с опозданием и поэтому отдельные точки непригодны для измерения времени. Вершина волны (v) показывает наименьшее опоздание в том случае, если кривая записывается с bulbus jugularis. Восходящая ветвь кривой (c), представляющая собой волну давления, появляется, независимо от расстояния от сердца, практический в одинаковое время. При помощи известного фотоэлектрического метода нельзя записать кривую с bulbus jugularis, только с помощью включения небольшого флажка. Методом авторов, основывающемся на измерении емкости, в большинстве случаев можно зарегистрировать хорошую механограмму также с bulbus jugularis и добиться максимального снижения времени опоздания объемных волн.

РЕЗУЛЬТАТЫ ДЛИТЕЛЬНОГО ЛЕЧЕНИЯ ХРОНИЧЕСКОГО ГЛОМЕРУЛОНЕФРИТА КОРТИКОИДАМИ

Й. БРОД, В. ФЕНЦЛ, З. ГЕЙЛ, Й. ЙИРКА и В. ПРАТ

1. 33 больных, страдавших по приведенным критериям весьма активным хроническим гломерулонефритом, и 5 больных со слабой активностью хронического гломерулонефрита подвергались длительному лечению кортизоном и преднизолом. 56 больных с высокой активностью и 65 больных со слабой активностью которые при впрочем одинаковом лечении не получали кортикоиды, служили контролем. Контрольная группа в отношении возраста, уровня почечной функции в начале наблюдения и направления развития клубочковой фильтрации, не различалась от подопытной группы. Группа с высокой активностью болезни в отношении продолжительности болезни также не показала отклонения от контрольной группы.

2. Под влиянием кортикоидов клинические симптомы активности болезни снижались как в группе с высокой, так и со слабой активностью болезни.

3. В группе с высокой активностью прекратилось ухудшение клубочковой фильтрации, и направление развития улучшилось в значительно более высоком проценте, чем у контрольной группы. В группе со слабой активностью болезни число больных, леченных кортикоидами, не было достаточным для статистической оценки.

4. Благоприятное действие лечения кортикоидами, как правило, продолжало проявляться также после прекращения курса лечения, и у преобладающей части больных за последующий период рецидивов не отмечалось.

5. В ходе лечения не наблюдалось серьезных осложнений.

ИССЛЕДОВАНИЕ ПОЧЕЧНОЙ ЛИМФЫ ПОСЛЕ ЗАКРЫТИЯ МОЧЕТОЧНИКА

М. ПАПП и М. Т. КОВАЧ

Автор экспериментально исследовал истечение почечной лимфы и ее состав в иннервированной и денервированной почках до и после закрытия мочеточника. Истечение лимфы из иннервированной и денервированной почек не показывает разницы. После закрытия мочеточника истечение лимфы из денервированной почки достоверно повышается ($p < 1\%$). По мнению автора лимфангиоспазм играет значительную роль в том, что после закрытия мочеточника истечение лимфы из иннервированной почки не повышается. Однако, если к закрытию мочеточника присоединяется застой в почечной вене, то истечение лимфы в этой подопытной группе также достоверно повышается ($p < 0{,}1\%$).

Под влиянием закрытия мочеточника содержание белков в лимфе во всех случаях достоверно снижается, как в подопытной группе с иннервированными почками ($p < 0{,}9\%$), так и в группе с денервированными почками ($p < 0{,}1\%$). При застое в почечной вене в группе с иннервированными почками содержание почечной лимфы, по сравнению с величинами, полученными после закрытия мочеточника, достоверно повышается ($p < 1\%$). Величина А/Г в почечной лимфе больше чем в плазме ($p < 1\%$). Осмотическая концентрация лимфы полученной как из иннервированной, так и из денервированной почек, постоянная — даже в случае выделения гипертонической мочи или после закрытия мочеточника. В группе животных с денервированными почками осмотическая концентрация плазмы, по сравнению с прочими подопытными группами, более низкая. В этой группе почечная лимфа — по сравнению с плазмой крови — осмотически гипертоническая. Застой в почечной вене не повлияет на осмотическую концентрацию почечной лимфы. Автор сообщает свою концепцию о связи между почечной функцией и лимфообращением в почках.

ЭКСПЕРИМЕНТАЛЬНЫЕ ИССЛЕДОВАНИЯ ИЗМЕНЕНИЙ, ПРОИСХОДЯЩИХ ВО ВНЕКЛЕТОЧНОМ ПРОСТРАНСТВЕ ПРИ ЛУЧЕВОМ СИНДРОМЕ

З. ЖЕБЁК и ДЬ. ПЕТРАНЬИ мл.

Авторы исследовали на крысах изменения внеклеточного пространства, наблюдаемые при желудочно-кишечном лучевом синдроме, вызванном облучением дозой 1500 г.

Он устанавливают, что в течение 24 часов после облучения состояние внеклеточного пространства весьма неустойчивое и показывает отклонения как в положительном,

так и в отрицательном направлениях. В этот период однократное определение изменений внеклеточного пространства не дает реальной картины. Начиная с 24-го часа, после развития желудочнокишечных симптомов, внеклеточное пространство постепенно уменьшается, а начиная с 65-го часа до смерти — внезапно уменьшается. За весь период наблюдения внеклеточное пространство уменьшилось на 54—58%.

Полученные результаты авторами обсуждаются в свете новых литературных данных о лучевом синдроме.

ТРАНССЕПТАЛЬНАЯ КАТЕТЕРИЗАЦИЯ ЛЕВОЙ ПОЛОВИНЫ СЕРДЦА

Г. Ш. КОВАЧ, Я. ПЕПО и Б. ФЕЛКАИ

Авторы подробно излагают технику транссептальной катетеризации левой половины сердца. Они применяли этот метод у 28 больных, — в 25 случаях с хорошим результатом. Существенных осложнений не наблюдалось. Описанная техника исследования надежна и просто проводима в любой кардиологической лаборатории. При помощи транссептальной катетеризации левой половины сердца можно получать хорошие кривые давления и точно диагносцировать патологические изменения левой половины сердца.

Авторы трактуют несколько характерных кривых давления.

ДИНАМИЧЕСКОЕ ИССЛЕДОВАНИЕ ЙОДОСОДЕРЖАЩИХ АМИНОКИСЛОТ СЫВОРОТКИ

И. РЕМЕНАР-БАЛОГ и П. КЕРТАИ

Дается описание простого метода для определения специфической активности йодосодержащих веществ крови. Экспериментально доказано расхождение соотношения аминокислот, содержащих J^{127} и J^{131}, циркулирующих в крови, и выявлено, что указанное отклонение меняется в зависимости от времени. Изменения можно отнести к тому факту, что аминокислоты с различным содержанием йода появляются в кровообращении в различные сроки, и остаются в кровяном русле в течение различного времени.

ДЕЙСТВИЕ ЭСТРИОЛА НА ОБМЕН ЛИПОИДОВ

М. ЮЛЕС, Б. М. ФРЁЛИХ, И. К. ЛАСЛО, И. ТОТ, Г. СЕПЕШШИ и М. А. ДАВИД

1) Авторы исследовали у 6 женщин различного возраста с различными диагнозами действие эстриола на концентрацию общих липоидов, фосфолипоидов, холестерина, холестерин-эфира, альфа- и бета-липопротеинов крови.

2) На основании результатов вышеприведенных исследований эстриол — в применяемых экспериментальных условиях — не оказывает существенного эффекта на уровень общих липоидов, фосфолипоидов, холестерина и холестерин-эфира.

3) Эстриол вызывает достоверное повышение содержания альфа-липопротеина в сыворотке и снижает концентрацию бета-липопротеина.

4) На основании полученных результатов авторы считают обоснованным испробовать противоатеросклеротическое действие биологически малоактивного эстрогена у мужчин с атеросклерозом или после перенесенного инфаркта миокарда.

ИССЛЕДОВАНИЕ ФУНКЦИИ ЩИТОВИДНОЙ ЖЕЛЕЗЫ ПРИ ДАЧЕ ПРОИЗВОДНОГО АЛКАЛОИДА СПОРЫНЬИ (БУТАНОЛАМИДА ЛИЗЕРГЕНОВОЙ КИСЛОТЫ)

Л. САНТО, А. Л. РЕВИЦКИ и Т. ГРИНЕУС

Авторы исследовали действие бутаноламида лизергеновой кислоты, антагониста серотонина, на функцию щитовидной железы. Результаты были оценены в опытах на животных на основе усвоения J^{131} щитовидной железой, при помощи анализа содержания

йода в щитовидной железе и в сыворотке методом бумажной радиохроматографии, далее in vitro на основании взаимодействия серотонина или десерила, добавленного к сыворотке крыс, инкубированной фосфатно-буферным раствором Кребс—Рингера, а также на основании терапевтических резуьлтатов на больничном материале. Бутаноламид лизергеновой кислоты во всех параметрах стимулировал функцию щитовидной железы:

1. повысилось усвоение J^{131} щитовидной железой,
2. повысилось содержание свободного T_4 и «conversion rate» в щитовидной железе крыс;
3. щитовидная железа выделяет повышенное количество T_4;
4. повышается периферическая дейодинация (как in vivo так и in vitro);
5. косвенные исследования (Десерил + MTU) говорят за повышение секреции TSH;
6. гистологические исследования указывают на постепенное развитие гиперфункции и
7. в экспериментах на людях наблюдаются у здоровых лиц и у больных миксэдемой признаки повышенной функции щитовидной железы.

По мнению авторов Десерил оказывает свое действие на систему гипоталамус—гипофиз—щитовидная железа—периферия путем

а) повышения периферической дейодинации,

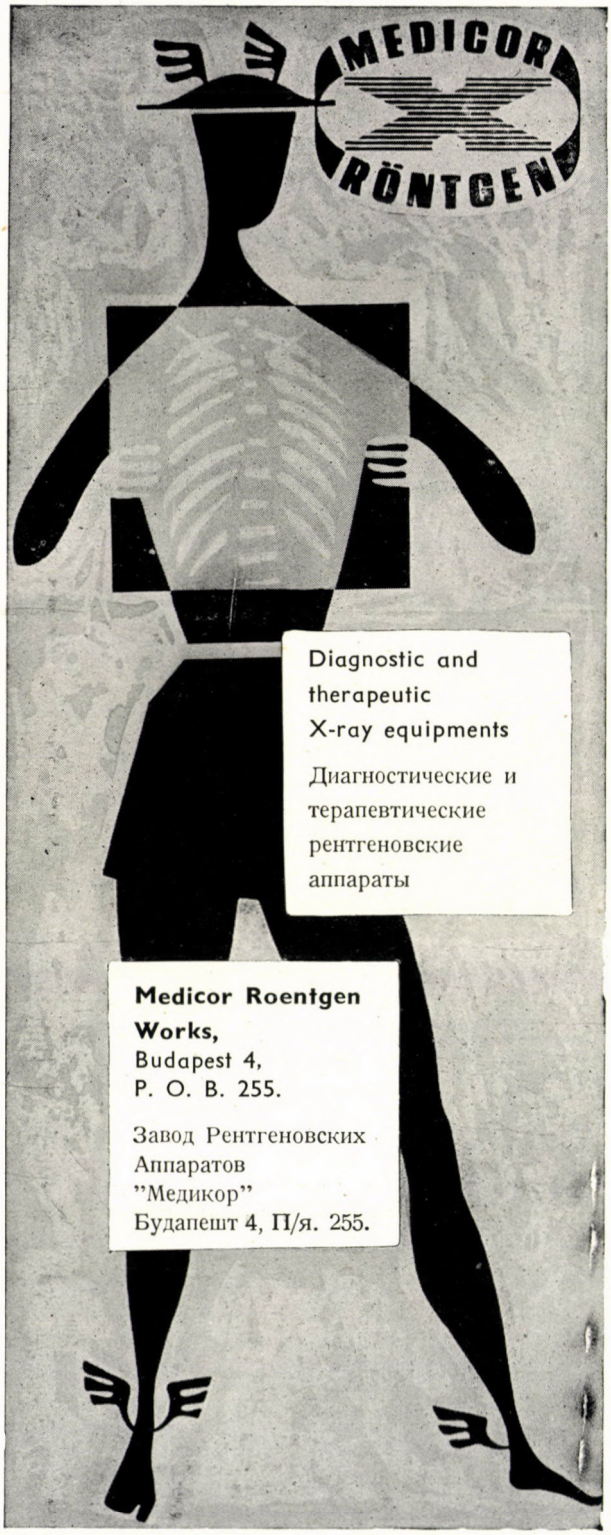
б) непосредственного стимулирования щитовидной железы и

в) косвенного стимулирования щитовидной железы посредством системы гипоталамус—гипофиз.

СВЯЗЬ МЕЖДУ КАПИЛЛЯРНОЙ ФИЛЬТРАЦИЕЙ И ЛИМФОТОКОМ ПРИ ВЕНОЗНОМ ЗАСТОЕ

ДЬ. САБО, Ж. МАДЬЯР и М. ПАПП

Авторы исследовали на задних конечностях наркотизированных собак действие венозного застоя на потерю жидкости и белков из капилляров, на лимфоток в конечностях и на уровень белков в лимфе. Было установлено, что между фильтрацией жидкости и лимфотоком существует — хотя и не очень тесная — но достоверно положительная связь ($r = 0{,}29$; p 5%). Лимфоток повышается параллельно венозному давлению. Эта зависимость выражается регрессионным уравнением: $L = P \times 4{,}01 + 76{,}3$. Содержание белков в лимфе гораздо больше, чем исчисленная концентрация белков в фильтрате. Между двумя величинами не удалось выявить корреляции ($r = 0{,}02$).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UNTERSUCHUNGEN DES LYMPHADENOGRAMMS BEI BÖSARTIGEN GESCHWULSTKRANKHEITEN

Von

M. Szegvári und A. Ihracska

FRAUENKLINIK (DIREKTOR: PROF. DR. F. E. SZONTÁGH) DER MEDIZINISCHEN UNIVERSITÄT, SZEGED

(Eingegangen am 18. Juni 1962)

Es wird über die Erfahrungen bei 2 Patientinnen mit bösartiger Geschwulst berichtet. Die Bedeutung der Lymphadenographie in der Diagnostik und in der Auswahl der entsprechender Therapie wird betont.

Die klinisch allgemein anwendbare Methode der Lymphangio- und Lymphadenographie wurde von dem englischen Chirurgen Kinmonth [4] ausgearbeitet. Mit diesem Verfahren können die präfaszialen Lymphgefäße und Lymphknoten der Extremitäten röntgenologisch dargestellt werden. Eine wichtige Bedeutung ist der Auffüllung der unteren Extremitäten beizumessen, da auf diese Weise ein bedeutender Teil der inguinalen, pelvinen und lumbalen Lymphknoten röntgenologisch sichtbar wird. Während früher die Diagnose der erwähnten Lymphknoten lediglich durch die histologische Untersuchung des operativ entfernten Materials gestellt werden konnte, sind dank der Methode heute die röntgenographischen Charakteristika der primären und sekundären Erkrankungen der Lymphknoten, in erster Reihe der bösartigen Geschwulste auf Grund der eingehenden Arbeiten von Colette [1], Kaindl [3], Fuchs [2] usw. bereits wohl bekannt.

In einer früheren Arbeit [5] haben wir über unsere Erfahrungen mit wäßrigem (Na-Azetriozat = Opacoron) und öligem (Lipiodol ultrafluid) Kontrastmittel berichtet. Die Untersuchungen wurden nur bei entsprechender Indikation durchgeführt.

Kasuistik

Fall 1. Frau J. R. (Nr. 181/62). Bei der 65jährigen Patientin wurde am 10. Jan. 1961 an einer gynäkologischen Abteilung eine intrauterine Untersuchung unternommen. Histologischer Befund: Adenocarcinoma corporis uteri. Behandlung: Abdominale Hysterektomie und Adnexentfernung, nachher perkutane Röntgenbestrahlung mit 8000 r. Ein Jahr nach der Operation klagte Patientin über Schmerzen, die in Richtung der beiden unteren Extremitäten ausstrahlten. Außerdem berichtete sie, daß zeitweise beide unteren Extremitäten anschwellen und daß an der Scham im Bereich des Scheidenmundes ein Knoten zu fühlen ist.

Die Untersuchung zeigte neben dem Orificium externum urethrae ein haselnußgroßes und im Scheidenstumpf ein nußgroßes, leicht blutendes nekrotisches Geschwulstgewebe. An den unteren Extremitäten, besonders am rechten Fuß ausdrückliches Ödem.

Die vorderen präfaszialen Lymphgefäße der beiden unteren Extremitäten wurden mit Lipiodol ultrafluid an aufeinanderfolgenden Tagen aufgefüllt. Am Lymphadenogramm

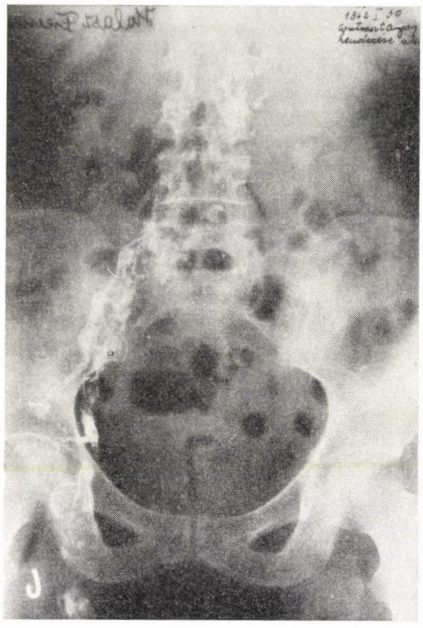


Abb. 1. Normales Lymphgefäß- und Lymphknotenbild. Inguinale, pelvine Lymphknoten, Lymphgefäßnetze. Auffüllung von der rechten unteren Extremität

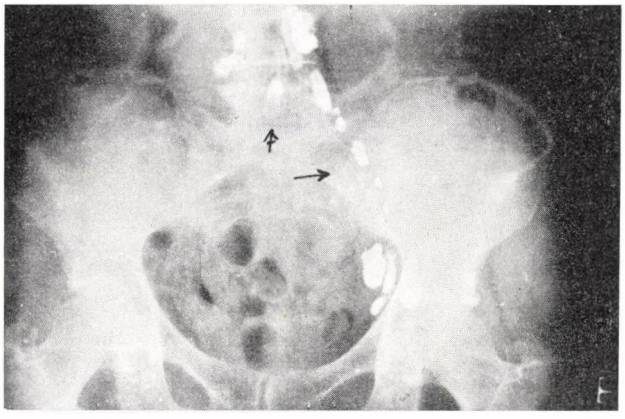


Abb. 2. Pelvine Lymphknoten. Auffüllung von der linken unteren Extremität. Keine Füllung der Lggl. iliacae ext. med. (→), mangelhafte Füllung mit Kontrastmittel des unteren Pols des oberen sakralen Lymphknotens (+→)

sind folgende Veränderungen vom Normalbild (Abb. 1) zu verzeichnen (Abb. 2): Partiale Füllung der Lggl. iliacae ext. med. Über das Promontorium scharfrandiger Schattenausfall im kaudalen Pol des in der Verzweigung der A. iliaca comm. liegenden solitären Lymphknotens, blasse Füllung im oberen Pol des in gleicher Höhe liegenden die A. iliaca ext. folgenden lateralen Lymphknotens. Auf Abb. 3 ist an einem bedeutenden, dem Verlauf der A. iliaca ext. entsprechenden Bereich keine Lymphknotenfüllung zu sehen. An dieser Stelle

ist ein ziemlich erweitertes S-förmiges Lymphgefäßbündel sichtbar, kranial davon sind kleinere Lymphknoten als üblich zu erkennen. Auch diese Aufnahme zeigt den, auch auf dem vorigen Bild sichtbaren scharfrandigen Schattenausfall im unteren Pol des Lymphknotens. Unseres Erachtens steht das Lymphangio- und Lymphadenogramm in Übereinstimmung mit den Beschwerden der Patientin und mit den klinischen Symptomen. Das Ödem der unteren Extremität ist Folge der Destruktion der pelvinen Lymphknoten. Das größere Ödem an der rechten unteren Extremität ist mit der ausgeprägten tumorösen Destruktion der rechtsseitigen Lymphknoten zu erklären. Die in die Schenkel ausstrahlenden Schmerzen können als Nervenkompressionssymptome infolge der Tumorinfiltration der pelvinen Lymphknoten betrachtet werden.

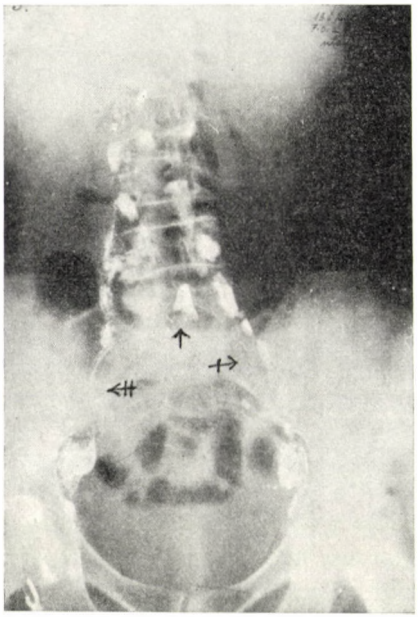


Abb. 3. (→) Scharfrandiger Schattenausfall. (+→) Keine Füllung der medialen Lymphknotenkette. (←+) Erweitertes Lymphgefäß an der Lymphknotenkette. Auffüllung von den unteren Extremitäten. Die Aufnahme wurde unmittelbar nach der Auffüllung der rechten und 24 Stunden nach der linken Seite verfertigt

Wir sind der Ansicht, daß die Durchführung der Lymphadenographie bei bösartige Geschwülsten im kleinen Becken dem Arzt in der Beurteilung des weiteren Bestrahlungs programms Hilfe leisten kann.

Fall 2. Frau Gy. M. (280/62). Klinische Aufnahme der 75jährigen Frau am 1. Febr. 1962. Anamnese: Seit 2 Monaten Ödem an der ganzen rechten unteren Extremität, so daß der Umfang doppelt so groß ist als der der linken unteren Extremität. Patientin bemerkte vor 6 Monaten einen nußgroßen Knoten in der Leistengrube, der sich durch Behandlung mit Umschlägen schnell zurückentwickelte. Zeitweise war sie fiebernd. Befund bei der Aufnahme: Senile Involution der äußeren und inneren Genitalien, im rechten subinguinalen Bereich unter dem ligamentum Pouparti zusammenhängende, nußgroße und kleinere, kaum bewegbare Lymphknotenbündel.

Diagnose: Lymphosarkom, Lymphadenitis, Metastasen in den Leistenlymphknoten.

Das qualitative Blutbild zeigte keine Leukämie an.

Lymphographie der rechten unteren Extremität (Abb. 4—6): Die präfaszialen vorderen medialen Bündel zeigen einen geraden Verlauf, normale Zahl der Bahnen (8—12). Suprapatellar ist ein blaßes, spinnengewebeartiges Fasersystem zu erkennen, das mit der retrograden Füllung der Lymphbahnen zu erklären ist. Geringe Verdickung der afferenten Lymphgefäße vor der Einmündung in die inguinalen oberflächlichen unteren Lymphknoten,

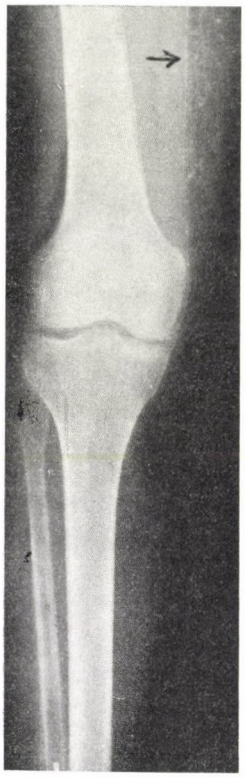


Abb. 4. (→) Geringe retrograde Füllung. Winzige Kontrastmittelextravasate

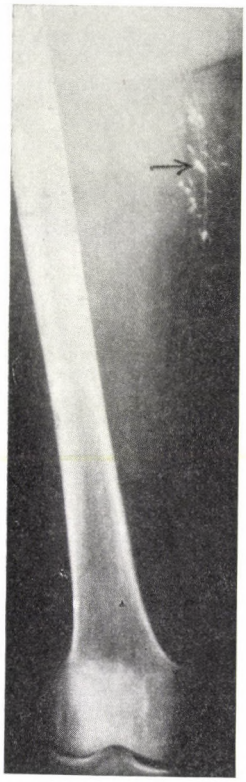


Abb. 5. (→) Die afferenten Lymphgefäße der oberflächlichen inguinalen unteren Lymphgefäße vor der Einmündung, außerdem größere Kontrastmittelextravasate

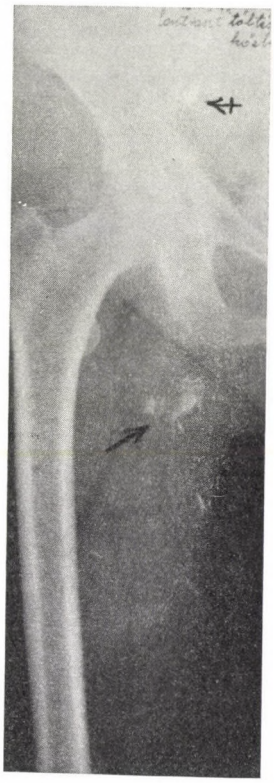


Abb. 6. (→) Destruktion des kranialen Teils der Lggl. inguinales superfic. inf. (→) Vergrößerter pelviner Lymphknoten

daselbst auch perilymphvaskuläre kleinere Kontrastmittelextravasate. Der Austritt des Kontrastmittels kann auf eine schwere Lymphgefäßwandentzündung (Lymphadenitis acuta) zurückgeführt werden. Das Lymphangiogramm zeigt ein pathologisches Bild der drei subinguinalen Lymphknoten: Das Kontrastmittel ist nur subkapsulär — bei der Einmündung der afferenten Bahnen — zu erkennen, die Lymphknoten zeigen Becherform, keine Auffüllung der efferenten Gefäße und der tiefen inguinalen Lymphknoten, Vergrößerung des unteren pelvinen Lymphknotens (Abb. 6).

Auf Grund des Lymphangiogramms wurde die Erkrankung des Lymphknotensystems angenommen, das Lymphangiogramm unterstützte das klinische Bild der Lymphangitis. Der operative Eingriff wurde nach der Analyse des Lymphadenogramms durchgeführt. Pathohistologische Diagnose: Lymphosarkom.

Die obigen Falldarstellungen sollen die Aufmerksamkeit auf die klinische Bedeutung der Lymphographie und Lymphadenographie lenken. Unsere Fälle beweisen, daß mit Hilfe der Lymphadenographie die primär tumorösen Erkrankungen und die Metastasen nachzuweisen sind. Auch in der Beurteilung der Tumorausbreitung ist diesem Verfahren Bedeutung beizumessen.

Wir sind der Ansicht, daß die Lymphadenographie als Hilfsmittel in der präzisen Diagnosestellung und in der Beurteilung der Propagation der tumorösen Veränderungen im kleinen Becken einen weiteren Fortschritt bedeutet. Außerdem bietet sie auch Hilfe in der chirurgischen und Strahlentherapie.

LITERATUR

1. Colett, J. M.: Acta radiol. (Stockh.) **49**, 154 (1958).
2. Fuchs, W. A., Böök-Hederström, G.: Acta radiol. (Stockh.) **56**, 340 (1961).
3. Kaindl, F., Mannheimer, E., Pflege-Schwarz, L., Thurnke, B.: Lymphographie und Lymphadenographie der Extremitäten. Thieme, Stuttgart, 1960.
4. Kinmonth, J. B.: Clin. Sci. **11**, 13 (1952).
5. Szegvári, M., Ihracska, A.: Magy. Radiol. **15** 72 (1963)

Dr. Menyhért Szegvári, Dr. Antal Ihracska
Frauenklinik der Medizinischen Universität, Szeged, Ungarn

ÜBER DEN MECHANISMUS DER ULZEROGENEN WIRKUNG DES BUTAZOLIDINS

Von

L. CSALAY und EDIT TÓTH

PATHOPHYSIOLOGISCHES INSTITUT (DIREKTOR: PROF. DR. J. SÓS) DER MEDIZINISCHEN UNIVERSITÄT, BUDAPEST

(Eingegangen am 24. Juli 1962)

Es wurde experimentell nachgewiesen, daß durch Verabfolgung verschiedener Butazolidin-Dosen die auf die Nebennieren bzw. den Magen ausgeübte Wirkung des Pharmakons differenziert werden kann. Bei täglicher Verabfolgung von 100 mg/kg Butazolidin treten die Magenschädigungen vor dem Anstieg der Kortikosteron-Synthetisierungsfähigkeit der Nebennieren auf. Eine Aktivierung der Nebennieren kann nur bei Verabfolgung größerer Butazolidin-Dosen — 200 mg/kg — nachgewiesen werden. Die Beobachtungen sprechen dafür, daß im Entstehen des ulzerogenen Butazolidin-Effekts die Funktionsveränderung der Nebennieren keine entscheidende Rolle spielt.

Des weiteren wurde der Butazolidin-Effekt auf die durch Histamin bewirkte Salzsäuresekretionssteigerung untersucht. 10tägige Butazolidin-Behandlung (150 mg/kg) steigert die Sekretion, werden jedoch größere Dosen verabfolgt (220 mg/kg), kann der Effekt nur am Anfang der Behandlung (5. Tag) nachgewiesen werden. Am 10. Tag der Behandlung sind die Werte der Salzsäuresekretion bei behandelten- und Kontrolltieren übereinstimmend. Das Aufhören der Salzsäuresekretionssteigerung am Ende der Behandlung kann teils mit der Toxizität des Butazolidins, teils mit dem Schleimhautödem erklärt werden.

Das salzsäuresekretionvermindernde Chlorothiazid konnte die ulzerogene Wirkung des Butazolidins nicht beeinflussen.

Klinische und tierexperimentelle Angaben beweisen eindeutig, daß das Butazolidin nebst seiner ausgezeichneten antirheumatischen Wirkung auch über ulzerogene Eigenschaften verfügt [1, 2, 3]. Der Mechanismus dieser Erscheinung ist trotz aller diesbezüglichen Forschungen noch nicht geklärt. Die Frage wurde von mehreren Seiten angenähert, so wurde z. B. die Wirkung des Butazolidins auf die Salzsäuresekretion [4, 5, 6, 7], und auf die innersekretorischen Drüsen untersucht [4, 12, 13, 14, 15]. VARRÓ, CSERNAY und JÁVOR [8] lenkten die Aufmerksamkeit auf das Ödem der Magenschleimhaut, ARON und LEFREIN [9] konnten die unmittelbar auf die Magenzellen ausgeübte toxische Wirkung des Butazolidins nachweisen. Die Ergebnisse sind jedoch so widersprechend, daß die sich auf den Pathomechanismus beziehenden Untersuchungen noch keineswegs als abgeschlossen betrachtet werden können.

Das Ziel vorliegender Experimente war die Beantwortung folgender Fragen:

1. Die Wirkung chronischer Verabfolgung verschieden hoher Dosen Butazolidins auf die Hormonsynthese der Nebennieren bei Ratten.

2. Der etwaige Zusammenhang zwischen der Nebennierenwirkung des Butazolidins und seinem ulzerogenen Effekt.

3. Die Beeinflussung der durch Histamin ausgelösten Steigerung der Salzsäuresekretion durch verschiedene Butazolidin-Dosen.

4. Der Einfluß von Chlorothiazid auf den ulzerogenen Effekt des Butazolidins.

Methodik

Ratten wurde täglich intraperitoneal 10 und 20 mg/100 g Butazolidin (Pharmazeutische Werke RICHTER, Budapest) verabfolgt. Am 5. und 10. Tag der Behandlung wurde die hormonsynthetisierende Fähigkeit der Nebennieren in vitro bestimmt und zwar stets 24 Stunden nach der letzten Injektion.

Prüfung der Hormonsynthese der Nebennieren: Beide Nebennieren einer Ratte wurden in ein 3 ml Tyrode-Lösung enthaltendes Warburg-Gefäß gelegt, 1 E ACTH (Exacthin, Pharmazeutische Werke Richter, Budapest) hinzugefügt, oxygenisiert und 2 Stunden lang in einem Wasserbad von 37 °C inkubiert. Die während dieser Zeit synthetisierte Hormonmenge wurde bestimmt. Das Corticosteron wurde mit Äthylacetat extrahiert, zur Extraktion wurde eine alkalische Lösung beigefügt [10, 21], die ausgelösten Stoffe wurden entfernt, die Alkalien mit Wasser ausgewaschen, und die Lösung mit Na_2SO_4 entwässert und verdampft. Der Rückstand wurde zwecks weiterer Reinigung mit Petroläther versetzt und schließlich — um die Oxydation der Steroide zu verhindern — unter Durchströmung mit CO_2 abermals verdampft. Der Extrakt wurde im BUSHschen System [10] in Toluol: Methanol : Wasser 4 : 3 : 1 chromatographiert und der Papierstreifen mit Tetrazoliumblau entwickelt. Die Formasan-Flecke wurden ausgeschnitten, die Farbe wurde eluiert, sodann ihre Intensität mit dem Stufenphotometer mit S 53-Filter bestimmt. Anläßlich jeder einzelnen papierchromatographischen Entwicklung wurden parallel mit dem Versuchsmaterial mindestens drei Corticosteron-Standarde von bekannter Konzentration mitentwickelt. Der Corticosterongehalt der unbekannten Substanz wurde aus der Eichkurve dieser stets am selben Papierstreifen entwickelten Corticosteron-Standarde von bekannter Konzentration berechnet. Im Verlaufe des Extraktions- und Reinigungsprozesses gehen 15% des Corticosterongehaltes verloren. So können also aus der mit peripherem Blut versetzten Corticosteronlösung von bekannter Konzentration 85% zurückgewonnen werden. Die Empfindlichkeit des Verfahrens beträgt bei Corticosteron 0.4 μg.

Das Gewicht der Nebennieren wurde pro 100 g Körpergewicht angegeben.

Salzsäuresekretionsbestimmung nach HERR und PÓRSZÁSZ (11): In den Pylorus der mit Urethan narkotisierten Tiere wurde eine Kanüle gebunden, dann mit Hilfe einer Schlundsonde der Magen zweimal mit je 5 ml physiologischer Kochsalzlösung durchgespült. Diese Prozedur wurde 2 Stunden später wiederholt. Von diesem Zeitpunkt an wurde der Magen stündlich mit 4 ml NaCl-Lösung durchgespült und die Azidität des Perfusates mit 0,01 n-NaOH titriert. Das Histamin wurde nach 2× 1stündiger Grundperiode verabreicht.

Die Ratten erhielten täglich 5 mg/kg Chlorothiazid per os.

Experimenteller Teil

Im ersten Teil der Experimente wurde die Wirkung verschiedener Butazolidin-Dosen auf die Nebennierenfunktion untersucht. Die bei den Bestimmungen der Corticosteron-Synthetisierungsfähigkeit gewonnenen Daten ließen den funktionellen Zustand der Nebennieren erkennen, diese Angaben wurden sodann mit der magenschädigenden Wirkung des Pharmakons verglichen.

Ratten wurde täglich 100 mg/kg Butazolidin verabfolgt. Am 11. Tag, 24Stunden nach der letzten Injektion wurden die Tiere dekapitiert. Es wurde in vitro untersucht wieviel Corticosteron die Nebennieren auf Wirkung

von 1 E ACTH in 2 Stunden synthetisieren. Die Ergebnisse sind in Abb. 1 zusammengestellt. Am rechten Graphikon der Abb. 1 ist die auf 100 g Körpergewicht berechnete Corticosteronsynthese von je 10, mit Butazolidin behandelten bzw. Kontrolltieren dargestellt. Aus der statistischen Analyse der Angaben ergibt sich, daß zwischen den beiden Gruppen keine signifikante Differenz besteht. Am linksseitigen Graphikon der Abb. 1 ist das auf 100 g Körpergewicht berechnete Nebennierengewicht angeführt. Die Zusammenstellung zeigt, daß auf Wirkung von Butazolidinbehandlung das Gewicht der Nebennieren

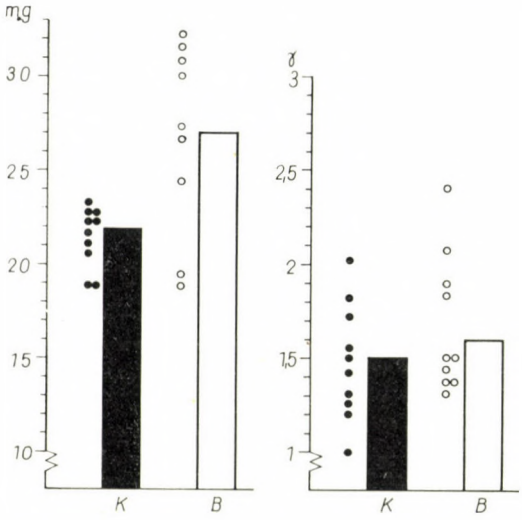


Abb. 1. Wirkung chronischer Butazolidin-Dosierung [100 mg/kg] auf Gewicht und Corticosteronsynthese der Nebennieren

Die Ordinate des linksseitigen Graphikons zeigt das auf 100 g Körpergewicht berechnete Nebennierengewicht in mg ausgedrückt. Die Ordinate des rechtsseitigen Graphikons bedeutet die auf 100 g Körpergewicht berechnete Corticosteronsynthese in μg. Die Punkte bedeuten die Werte der einzelnen Tieren, die Kolumnen enthalten die Gruppendurchschnittswerte. K = Kontrolle, B = Butazolidin

zunahm, die Experimente beweisen jedoch, daß das in identischen Mengen verabfolgte Butazolidin keine Erhöhung der Nebennierenhormonsynthese bewirkte. In der Magenschleimhaut der behandelten Tiere waren makroskopische Blutungen sichtbar. Die Veränderungen betreffen den sekretorischen Magenteil. Intakter Vormagen. Die Veränderungen sind in Abb. 2 dargestellt. Laut der histologischen Untersuchung entsprechen die im Magen befindlichen Veränderungen hämorrhargischen Erosionen.

Auf Grund der Experimente kann festgestellt werden, daß kleine Butazolidindosen, die im Magen bereits Erosionen verursachen, die Nebennierenhormonsynthese nicht steigern, im ulzerogenen Effekt des Butazolidins spielen also die Nebennieren keine wesentliche Rolle.

Des weiteren wurde die Wirkung größerer Butazolidindosen auf die Nebennierenfunktion untersucht. Ratten wurde während 5 bzw. 10 Tage täglich 200 mg/kg Butazolidin verabfolgt und die Corticosteron-Synthetisierungsfähigkeit in ähnlicher Weise bestimmt. Die Ergebnisse sind in Abb. 3 dargestellt. Sowohl die 5- als die 10tägige Behandlung führt zu signifikanter Steigerung der Hormonsynthese, sowie zu Erhöhung des Nebennierengewichtes. Die Experimente zeigen somit, daß diese großen Butazolidindosen die Nebennierenfunktion bereits steigern. Infolge der bekannten ulzerogenen Wirkung des Nebennierenrindenhormons kann das Butazolidin — annehmbar auch auf

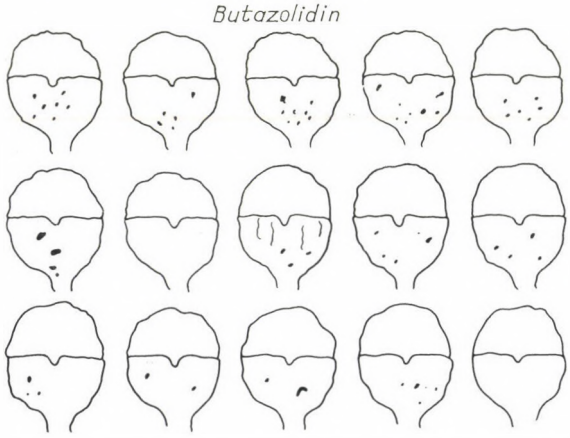


Abb. 2. Magenschleimhaut der Ratten nach 10tägiger Butazolidin-Behandlung: Dosierung 100 mg/kg/Tag

Die Bilder zeigen die Konturen des entlang der großen Kurvatur aufgeschnittenen und ausgebreiteten Magens. Oben befindet sich der Vormagen, die im unteren sekretorischen Magenteil sichtbaren schwarzen Punkte und Linien zeigen Größe und Anzahl der hämorrhagischen Erosionen

diese Weise — auf die Magenschleimhaut einwirken, es soll jedoch betont werden, daß in der Magenschleimhautschädigung noch einem anderen wesentlichen Wirkungsmechanismus eine Rolle zukommt, da — wie dies die Experimente bewiesen — diese unmittelbare Wirkung im ulzerogenen Effekt des Butazolidins nur unwesentlich sein kann.

Da durch entsprechende Butazolidindosierung die Magen- und Nebennierenwirkung des Pharmakons differenziert werden konnte, war das weitere Ziel, die widersprechenden Ergebnisse, die bei der Untersuchung des Butazolidin-Effekts auf die Salzsäuresekretion gewonnen wurden, mit Hilfe einer geeigneten Methodik bzw. durch Anwendung verschiedener Butazolidindosen zu klären.

Die Salzsäuresekretion wurde statt der üblichen Shayschen Technik mit der Perfusionsmethodik von Herr und Pórszász [11] bestimmt. Laut

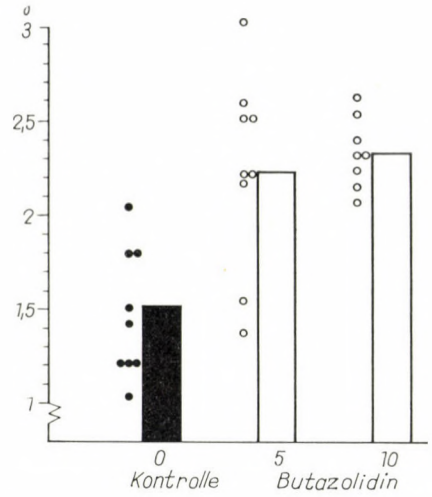


Abb. 3. Wirkung 5- bzw. 10tägiger Butazolidinbehandlung [200 mg/kg] auf die Corticosteronsynthese der Rattennebennieren. Ordinate: Die in 2 Stunden auf ACTH-Wirkung in vitro synthetisierte Corticosteronmenge in µg/100 g Körpergewicht. Abszisse: Tage der Butazolidin-Behandlung
Bedeutung der Punkte und Kolumnen s. Abb. 1

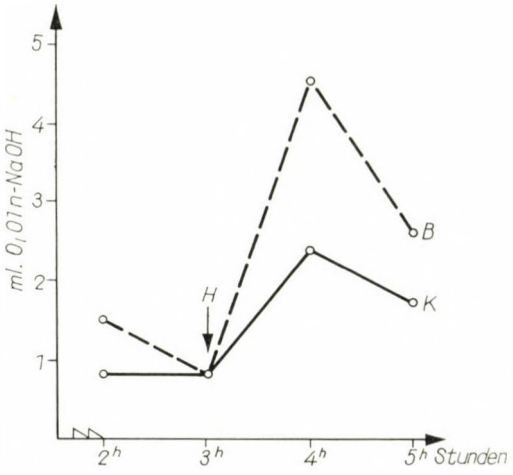


Abb. 4. Wirkung 10tägiger 150 mg/kg Butazolidin-Behandlung auf die durch Histamin gesteigerte Salzsäuresekretion
An der Abszisse befinden sich die Zeitpunkte der Magenspülungen in Stunden (Punkt 0 bedeutet den Zeitpunkt der Kanüleeinführung). Ordinate: Die durch das Perfusat verbrauchten ml der 0,01 n-NaOH-Lösung. Die kontinuierlichen bzw. gestrichelten Linien zeigen die Durchschnittswerte der Salzsäuresekretion der Kontroll- bzw. der behandelten Tiere. Die Differenz zwischen den beiden Gruppen ist statistisch signifikant: $p < 0{,}01$

unserer Untersuchungen bedeutet diese Methodik für die Tiere eine kleinere Belastung und liefert genauere Ergebnisse als das SHAYsche Verfahren.

Ratten wurde zuerst intraperitoneal 150 mg/kg Butazolidin verabfolgt. Am 10. Tag wurde der auf 10 mg/kg Histaminwirkung erfolgende Salzsäuresekretionsanstieg im Magen der behandelten und Kontrolltieren untersucht. Die in Abb. 4 dargestellten Ergebnisse zeigen, daß bei den behandelten Tieren nach Histamingabe eine wesentlichere Steigerung der Salzsäuresekretion erfolgt als bei den Kontrolltieren. Die Differenz ist statistisch signifikant ($p < 0{,}01$).

In der nächsten Serie wurde die Wirkung von 220 mg/kg Butazolidin untersucht. 13 der 20 behandelten Tiere gingen während der 10tägigen Behandlung ein, es handelte sich also um eine hypertoxische Dosis. Abb. 5 enthält die

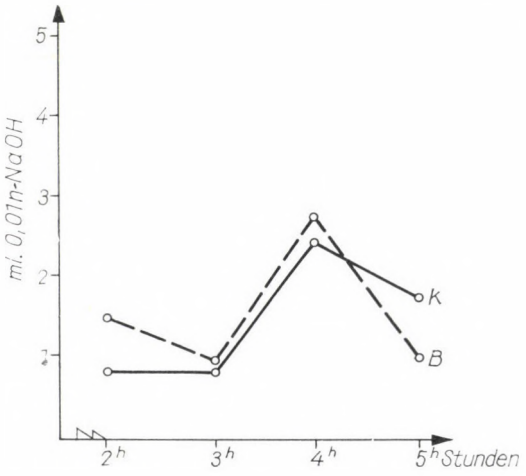


Abb. 5. Wirkung 10tägiger Butazolidin-Behandlung [220 mg/kg] auf die durch Histamin gesteigerte Salzsäuresekretion. Text s. Abb. 4

Ergebnisse der an diesen Tieren vorgenommenen Salzsäuresekretionsuntersuchungen: Auf Histaminwirkung ist die Salzsäuresekretion die gleiche wie bei den Kontrolltieren, die bei Verabfolgung kleinerer Butazolidindosen beobachtete Steigerung kommt also im Fall von Überdosierung nicht zustande. Makroskopisch war die Magenschleimhaut der Ratten ödematös, im sekretorischen Magenteil waren die bekannten Erosionen sichtbar.

Um die Dynamik der Erscheinung zu untersuchen, wurde die auf Histaminwirkung zustandekommende Salzsäuresekretion auch am 6. Tag der letzterwähnten experimentellen Serie bestimmt. Das Ergebnis stimmt mit den bei 150 mg/kg Butazolidin-Verabfolgung gewonnenen Resultaten überein, d. h. die behandelten Tiere sezernieren mehr Salzsäure als die der Kontrollgruppe. Da die säuresekretionsteigernde Wirkung des Histamins nach mehrtägiger Verabfolgung dieser Dosis nicht wahrzunehmen ist, konnte während der lang-

dauernden Behandlung die salzsäuresekretionsteigernde Wirkung des Histamins, entweder wegen der ödematösen Schleimhaut oder wegen der im Stoffwechsel der Mukosa zustandekommenden Veränderungen scheinbar nicht zur Geltung kommen.

In Kenntnis der die salzsäuresekretionsteigernden Wirkung der Butazolidin-Behandlung ergab sich die Frage, welche Rolle das Pharmakon im ulzerogenen Effekt spielt. Um dem Problem näher zu kommen, wurde in weiteren Experimenten untersucht, ob die magenschädigende Wirkung des Butazolidins durch Verabfolgung von 5 mg/kg die Salzsäuresekretion verminderndem Chlorothiazid gehemmt werden kann. Zwei, je 10 Ratten enthaltenden Gruppen wurde während 10 Tage täglich 150 mg/kg Butazolidin intraperitoneal verabfolgt. Den Tieren der ersten Gruppe wurde außerdem 5 mg/kg Chlorothiazid verabreicht, die zur 2. Gruppe gehörenden Tiere dienten als Kontrolle. Im sekretorischen Magenteil der am 10. Tag getöteten Ratten waren in beiden Gruppen die gleichen Läsionen zu erkennen, der ulzerogene Effekt des Butazolidins konnte also durch die Behandlung nicht beeinflußt werden.

Diskussion

Bazzi und Manzo [15] waren bereits 1952 der Meinung, daß das Butazolidin die Nebennierenfunktion steigert. Während die Beobachtung von Christeas [24] — daß nämlich anläßlich der Butazolidin-Behandlung Eosinopenie auftritt — diese Hypothese unterstützt, konnten Kirsner und Ford [4] bei mit Butazolidin behandelten Patienten weder Nebennierenhyperplasie, noch gesteigerte 17-Ketosteroid-Ausscheidung wahrnehmen. Die tierexperimentellen Angaben sind ebenfalls widersprechend: Kuzell und Mitarb. [14] konnten in akuten Versuchen, nach Butazolidin-Behandlung keine Veränderung des Vitamin-C Gehaltes der Nebennieren feststellen, Braun [13] fand dagegen bei Ratten nach Verabfolgung ulzerogener Butazolidin-Dosen Verminderung des Vitamin-C-Gehaltes der Nebennieren und Eosinopenie. Nach chronischer Butazolidin-Verabfolgung beobachtete Eger [12] außer den ulzerösen Magenerscheinungen, Nebennierenhypertrophie und Zunahme des Lipoidgehaltes in allen drei Rindenschichten. Er war der Meinung, daß es sich um Adaptationserscheinungen handelt.

Neuere experimentelle Angaben bewiesen, daß nebst der in Ulkusversuchen bisher angewandten chronischen Verabfolgungsweise weder die Veränderungen des Vitamin-C-Gehaltes der Nebennieren, noch die Veränderungen der Eosinophilenzahl ein zuverläßliches Bild der Nebennierenfunktion ergeben. Unsere Experimente [16, 17] zeigen, daß bei verschiedenen Belastungen ausgesetzten Tieren weder der Corticosterongehalt des venösen Nebennierenblutes noch die Hormon-Synthetisierungsfähigkeit der Nebennieren der Gestaltung des histologischen Bildes parallel verlaufen. Die Zunahme des

Nebennierengewichtes deutet ebenfalls nicht genau auf den funktionellen Zustand der Organe, wie das auch unsere vorangehenden Experimente beweisen: Bei an Eiweiß- und Methionin-Mangeldiät gehaltenen Tieren [16, 23] wurde bei normalem Nebennierengewicht, bei Ratten mit Triptophanmangel nebst hypertropischen Nebennieren eine verminderte Hormonsekretion beobachtet. Bei den systematisch zu Schwimmen gezwungenen Tieren konnte zwischen Gewicht und Synthetisierungsfähigkeit der Nebennieren ebenfalls keine Parallelität festgestellt werden [17]. Im vorliegenden Experiment wurde eben deswegen aus dem direkten Hormonbestimmungsverfahren auf den funktionellen Zustand der Nebennieren gefolgert.

Unsere Experimente beweisen, daß durch Verabfolgung verschiedener Butazolidin-Dosen die auf Nebennieren bzw. Magen ausgeübte Wirkung des Pharmakons differenziert werden kann. Wird das Butazolidin in kleinen täglichen Dosen verabfolgt, so entwickeln sich die Magenschädigungen vor der Steigerung der Corticosteron-Synthetisierungsfähigkeit der Nebennieren. Die Aktivierung der Nebennieren manifestiert sich nur bei größeren Butazolidin-Dosen. Diese Beobachtungen sprechen dafür, daß im Zustandekommen des ulzerogenen Butazolidin-Effekts den Funktionsveränderungen der Nebennieren keine wesentliche Rolle zukommt. Die Erklärung der widersprechenden Literaturangaben liegt wahrscheinlich darin, daß die Forscher teils verschiedene Butazolidin-Dosen verabfolgten, teils zur Funktionsprüfung der Nebennieren Teste verschiedener Empfindlichkeit angewendet haben.

Um den Mechanismus des ulzerogenen Butazolidin-Effekts zu klären, wurde des weiteren die auf die Salzsäuresekretion ausgeübte Wirkung des Pharmakons untersucht. Die Literaturangaben sind auch in bezug auf diese Frage widersprechend.

Während Kirsner und Ford [4] bei Menschen anläßlich chronischer Butazolidin-Verabfolgung die Steigerung der Salzsäuresekretion wahrnehmen konnten, fand Lambling [18] gemeinsames Entstehen von diffuser Gastritis und Hyperchlorhydrie. Laut Varró, Csernay und Jávor [8] erfolgten bei Hunden nach ulzerogener Butazolidin-Dosierung keine Veränderungen. Bei Meerschweinchen [6] bewirkt das Pharmakon eine Verminderung der Salzsäuresekretion [16]. Die an Ratten durchgeführten Experimente ergeben ebenfalls widersprechende Resultate: Verminderung [5] und Zunahme der Sekretion wurden gleichfalls beschrieben [19]. Die Tatsache, daß diese Angaben bei Tieren mit unterbundenem Pylorus gewonnen wurden, erschwert die Klärung der Frage, da das Butazolidin bekanntlich die Entwicklung des Shayschen-Ulkus beeinflußt [7]. Die Pylorusunterbindung veranlaßt nicht nur eine Steigerung der Salzsäuresekretion, sondern verursacht auch schwere Zirkulations-, organische und endokrine Störungen [20], die das Butazolidin infolge seiner antiphlogistischen, analgetischen, die Schilddrüsenfunktion vermindernden und die Nebennierenfunktion steigernden Wirkung beeinflussen kann.

Zwischen der durch Pylorusunterbindung verursachten Salzsäuresekretionssteigerung und den erwähnten organischen Veränderungen besteht ein enger Zusammenhang, so daß die unmittelbare Einwirkung des Pharmakons auf die Salzsäuresekretion mit dieser Methode nicht untersucht werden kann.

Eine weitere Schwierigkeit bedeutet, daß das infolge der Magenschleimhautverletzung in den Magensaft gelangende Blut die Salzsäuresekretion wesentlich stört. Die Vorteile des in unseren Experimenten angewandten Perfusionsverfahrens nach HERR und PÓRSZÁSZ [11] sind die folgenden: Teils ist der Sekretionsreiz nicht die Pylorusunterbindung, sondern das unmittelbar auf die Magenzellen einwirkende Histamin, teils kann die Bestimmung in kürzerer Zeit durchgeführt werden, der Magensaft gelangt wegen der wiederholten Spülungen nur kurz mit der Magenwand in Berührung, so daß die aus der Schleimhautschädigung stammende Blutung die Bestimmung weniger stört. Die mit dieser Methode vorgenommenen Versuche ergaben, daß die 10tägige Butazolidin-Verabfolgung mit 150 mg/kg die salzsäuresekretionsteigernde Wirkung des Histamins erhöht. Wird 220 mg/kg die gleiche Zeit lang dosiert, kann diese Wirkung nicht wahrgenommen werden. Letzteres steht wahrscheinlich mit der Toxizität des Butazolidins im Zusammenhang, da nur 7 der mit dieser Dosis behandelten Tiere am Leben blieben. Bekanntlich hemmt das Butazolidin zahlreiche Stoffwechselvorgänge, so daß annehmbar auch der Salzsäuresekretionsmechanismus gestört wird. Die Magenschleimhaut dieser Tiere war auch makroskopisch ödematös. VARRÓ, CSERNAY und JÁVOR [8] konnten bei Hunden ähnliche Erscheinungen beobachten. Werden die Tiere mit großen Butazolidin-Dosen kürzere Zeit behandelt, so kann die salzsäuresekretionsteigernde Wirkung des Histamins noch zur Geltung kommen.

Es erhebt sich die Frage, welche Rolle die gesteigerte Salzsäuresekretion im Mechanismus der ulzerogenen Wirkung des Butazolidins spielt. Experimentelle Daten beweisen, daß allein die Steigerung der Salzsäuresekretion noch kein Ulkus verursacht, und daß die erhöhte Sekretion für die Schädigung der Mukosa nur dann verantwortlich ist, wenn die Widerstandsfähigkeit der Magenschleimhaut vermindert ist. Laut Literaturangaben kommen nach Butazolidin-Verabfolgung im Stoffwechsel der Mukosa Veränderungen zustande. Theoretisch besteht also die Möglichkeit, daß im Entstehen der Schleimhautschädigung auch der gesteigerten Salzsäuresekretion eine Rolle zukommt. Nach experimentellen Daten konnte bei Meerschweinchen durch Neutralisation der Salzsäure das Zustandekommen der Magenschädigungen in 30% der Fälle verhindert werden [6]. In diesen Experimenten wurden interessanterweise die Salzsäuresekretion vermindernde Butazolidin-Dosen verabfolgt. VARRÓ und Mitarb. [8] konnten bei Hunden die ulzerogene Butazolidin-Wirkung mit die Salzsäuresekretion verminderndem Atropin nicht beeinflussen.

Wir untersuchten auch die Wirkung von Chlorothiazid. Dieses Pharmakon vermindert teils die Salzsäuresekretion, teils die durch Butazolidin ver-

ursachte Wasserretention, was sich in den Experimenten im Ödem der Mukosa manifestierte.

Aus den Versuchsergebnissen geht hervor, daß Chlorothiazid-Verabfolgung die ulzerogene Butazolidin-Wirkung nicht beeinflußt, es soll jedoch erwähnt werden, daß das Chlorothiazid die Salzsäuresekretion nicht vollkommen blockiert, sondern nur vermindert. Die Untersuchungen, die sich mit der Rolle der Salzsäure im Entstehen des Butazolidinulkus befassen, können also nicht als abgeschlossen betrachtet werden.

Es ist möglich, daß bei den mit Butazolidin behandelten Tieren die Salzsäuresekretionssteigerung nach Histamin nur eine Teilerscheinung des Reizzustandes der Schleimhaut ist, und daß im Mechanismus der ulzerogenen Wirkung nicht nur der Steigerung der Salzsäuresekretion, sondern vielmehr den Stoffwechselstörungen der Salzsäure sezernierenden Schleimhaut eine wesentliche Rolle zukommt.

LITERATUR

1. Maurer, E. F.: New Engl. J. Med. **253**, 404 (1955).
2. Raffensberger, E. C.: J. Amer. med. Ass. **152**, 30 (1953).
3. Krainin, P.: J. Amer. med. Ass. **152**, 31 (1953).
4. Kirsner, J. B., Ford, H.: Gastroenterology **29**, 1 (1955).
5. Bonfils, S., Hardouin, I. P., Richiz, Cl., Dalbare, Fl., Lambling, A.: Bull. Soc. méd. Hôp. Paris, **71**, 114 (1955.)
6. Varco, C.: Gastroenterology **37**, 87 (1959).
7. Bonfils, S., Hardouin, J. P., Dalbare, F. I.: C. R. Soc. Biol. (Paris) **148**, 881 (1954).
8. Varró, V., Csernay, L., Jávor, P.: Gastroenterology **37**, 463 (1959).
9. Aron, E., Lefrein, M., Jobard, P.: Arch. Mal. Appar. dig. **45**, 496 (1956).
10. Bush, I. E.: Biochem. J. **50**, 370 (1951).
11. Herr, F., Pórszász, J.: Acta physiol. Acad. Sci. Hung. **2**, 17 (1951).
12. Eger, W.: Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak. **224**, 1 (1954).
13. Braun, H.: Arch. int. Pharmacodyn. **101**, 381 (1955).
14. Kutzell, W. C., Schaffarzick, R. W., Brown, B., Mankle, E. A.: J. Amer. med. Ass. **149**, 729 (1952).
15. Bazzi, U., Mauro, F.: Boll. Soc. ital. Biol. sper. **29**, 12 (1953).
16. Csalay, L.: Dissertation, 1958.
17. Frenkl, R., Csalay, L., Döklen, A., Szepessi, E., Tóth, E.: Acta physiol. Acad. Sci. hung. Suppl. **16**, 103 (1959).
18. Lambling, A., Bonfils, S.: Rev. méd. franç. **36**, 60 (1955).
19. Kalliomaki, J., Keyrilainen, T. O., Gronroos, M.: Ann. Med. exp. Fenn. **35**, 446 (1957).
20. Lambling, A., Hardouin, J. P., Bonfils, S., Lamnouien, R.: Arch. Mal. Appar. dig. **42**, 417 (1953).
21. Vogt, M.: J. Physiol. (Lond.) **130**, 601 (1955).
22. Csalay, L., Frenkl, R., Döklen, A., Molnár, Zs., Somogyi, Gy.: Acta physiol. Acad. Sci. hung. Suppl. **16**, 101 (1959).
23. Csalay, L., Weisz, P., Sós, J., Frenkl, R.: Z. Vitamin-, Hormon- u. Fermentforsch. Im Druck.
24. Christeas, N., Dascalakis, T., Chrysospathis, P.: Arch. Mal. Appar. dig. **45**, 239 (1956).
25. Gross, F.: Klin. Wschr. **34**, 48 (1956).
26. Hiller, J.: J. exp. Med. **126**, 244 (1955).
27. Hiller, J., Strauss, E.: Medizinische **1**, 613 (1954).

Dr. László Csalay, Dr. Edit Tóth | Budapest IX. Hőgyes E. utca, Kórélettani Intézet, Ungarn

A NEW METHOD FOR THE DEMONSTRATION OF ENTEROTOXIN PRODUCTION BY STAPHYLOCOCCI

By

I. NIKODÉMUSZ, L. KANIZSAI and E. SÉLLEI

NATIONAL INSTITUTE OF NUTRITION, BUDAPEST, AND THE PÉCS–BARANYA COUNTY PUBLIC HEALTH-EPIDEMIOLOGICAL STATION, PÉCS

(Received September 22, 1962)

In connexion with food poisoning caused by *Staphylococcus aureus* we have attempted to demonstrate by a new method the enterotoxin production by the microorganisms isolated from food samples. Adult dogs were fed with the culture of the strain in question; the animals after a latency period of 4 to 5 hours responded with frequent diarrhoea and some other symptoms. Unlike in human disease, vomiting was infrequent. The symptoms persisted for 7 to 9 hours. From the stools large quantities of staphylococci could be grown for 8 to 12 hours, and small amounts for an additional 12 hours. The vomits, too, were positive for *Staphylococcus aureus*. Under similar conditions no disease could be induced by the feeding of apathogenic staphylococcus strains.

The method described, although less sensitive than Dolman's test, may lend itself for the demonstration of enterotoxin production by staphylococci.

The incidence of food poisoning of staphylococcal aetiology is different in the different countries. In the U. S. A. they cause 70 to 90 per cent [1], in Italy 24 to 28 per cent [2, 3], in France 24 per cent [4], in Great Britain 2 per cent [5] of food poisonings. In Hungary they are responsible for a considerable number; according to the statistical data of our Institute, in the period 1950 to 1956 they caused food poisoning in 10 per cent, in the period 1956—60 in 22 per cent of the cases [6]. The data for 1951—59, published by the Budapest Epidemiological Station, showed a 16 per cent incidence of staphylococcal aetiology [7]. These data make it obvious that the microorganism plays an important role in nutritional hygiene. To prevent the occurrence of food poisonings it is imperative that foodstuffs should be subjected to systematic bacteriological examinations and those which are found to contain larger quantities of staphylococci must be withheld from consumption. Many foodstuffs may, of course, contain Staphylococci, according to our unpublished data, small quantities of it (10^1—10^2/g) may be demonstrated in the microflora of 50 per cent of all foods. Naturally, it is not irrelevant whether the strain found in the food is pathogenic or non-pathogenic, because we formulate our opinion on the basis of pathogenicity, in the first place.

The different authors ascribe the general and enteral pathogenicity of staphylococci to different morphological and biological properties. It is universally accepted that the coagulase-positive *Staphylococcus aureus haemolyticus* strains are pathogenic, and, at the same time, it is these strains that produce

enterotoxin. Beside this property attention has been drawn to the property of breaking down mannitol [8], phosphat [9], lecithin [10], fats [11]. According to the general opinion, the more these so-called pathogenicity tests yield positive results, the more likely it is that the strain in question is pathogenic. KRINSKY et al. [11] examined in detail the fermentative activities of staphylococcus strains isolated from 1420 patients and healthy subjects, and concluded that certain enzymatic functions show a parallellism with the pathogenicity, but no single enzymatic activity would be a 100 per cent proof of pathogenicity. On the other hand, Rumanian authors have pointed out that there exist staphylococci, which do not give *in vitro* pathogenicity tests, but produce enterotoxin. Therefore they think it reasonable to test for enterotoxin production every strain of staphylococcus isolated from cases of food poisoning [12].

The test for the demonstration of enterotoxin was worked out by DOLMAN and WILSON [13] in 1940. Since then, it has been used extensively, but it may occur, as it has been reported for example by HUET [14] and BUTTIAUX [15], that a staphylococcus strain causing food poisoning is negative by the Dolman test. Other tests recommended for the demonstration of toxin production are the frog test [16], as well as the intracerebral inoculation of mice, but these methods are not extensively used [17].

KIENITZ [18] analysed in detail the possibilities of demonstrating staphylococcal enterotoxin, and arrived at the conclusion that, apart from the experiments involving human volunteers, only the tests made in young cats and monkeys are reliable, much more so as Richmond's rabbit gut test and Robinton's frog test.

Albino mice, guinea pigs and rabbits are not suitable for the demonstration of toxin and, as far as one can judge from the data published, it is impossible to reproduce staphylococcal intoxication even in the dog. MINETT was the first to administer through a gastric tube 20 to 40 ml of staphylococcal filtrate mixed with the same volume of milk to dogs 6 to 12 months of age. Some animals vomited and had diarrhoea, but later failed to respond with a similar reaction. By administering toxin parenterally, RIDGON [20] succeeded in producing symptoms of intoxication, while JORDAN did not [21]. We shall come back to these results later. SEDLAK [22] and KIENITZ [18] have arrived at the same conclusion concerning the evaluation of the various biological tests.

As already mentioned, in Hungary staphylococci often play a pathogenic role in food poisoning, and we consider it highly recommendable to make systematic tests for the demonstration of enterotoxin, as it was in fact done in many a case [23, 24]. The main obstacle of biological assay is that young cats are not always available. There is therefore a need for developing another, more feasible procedure. We have worked out a new method for the demonstration of enterotoxin in connexion with food poisoning involving a whole family. Before describing the method, we present the pertaining case reports.

In April, 1961, a wedding dinner took place, after which many of the guests developed food poisoning and 5 of them had to be taken into hospital. In the history of every one of them there was evidence that they had consumed food left over from the dinner. The symptoms began to appear 2 to 3 hours later. Acute gastric pains, nausea, vomiting developed suddenly, followed by frequent attacks of diarrhoea, first formed stools, then liquid, watery, and occasionally containing pus and blood. The patients had to be taken to a hospital owing to this severe condition. On admission the most conspicuous finding was the extreme weakness of the patients, some of whom lost consciousness, while others were near to collapse. Three patients recovered and were discharged 7 days, two of them 8 days after admission.

At the hospital first salmonellosis was suspected but no salmonella could be isolated from the faeces. Eight samples of food were sent to the Epidemiological Station; from five of them (fancy cake, dressed meat, cream pie, boiled ham, butter cookies) coagulase positive *Staphylococcus aureus haemolyticus* could be grown, whereas three other samples contained no pathogenic bacteria. It is to be noted that since the samples had been sent in after storage for a longer period of time, no detailed bacteriological tests were made, but from the above mentioned 5 samples the staphylococcus grew in almost pure cultures in blood agar and in pure cultures in media containing 8 per cent NaCl.

The clinical symptoms corresponded to those of staphylococcal food poisoning. As it turned out later, one member of the family had suffered from follicular tonsillitis two weeks before the dinner and at the time the foods were cooked every member of the family was complaining about common cold and angina. No tests of throat or sputum samples were made, but the foods might have been infected that way. Since only a small part of the foods was consumed within a short time after their preparation, in the stored foods the staphylococci had time to multiply and produce toxin in such amounts, as might explain the severe symptoms.

Five of these staphylococcus strains were sent to the Microbiological Department of the National Institute of Nutrition for enterotoxin tests. Although the five strains seemed to be identical, we tested them one by one. Mice were inoculated intracerebrally, on the one hand, and, on the other, sterile milk had been infected with cultures of the strains in question and 1 litre volumes of such 24-hour cultures were fed to 2—2 adult dogs (14 to 18 kg). At the time of feeding the milk samples contained 10^6 to 10^8/g living *Staphylococcus aureus* organisms. The animals, fasted moderately before, ingested the milk without fail within 20 to 60 minutes. In the following we describe the results obtained with the different strains.

Strain I (1.774/61). Four hours after having ingested the infected milk 1, five hours later 3, and ten hours later 2 liquid stools were passed. Between 7 and 8 hours one of the dogs vomited twice. Both animals were inert and had

no appetite. They seemed to feel better after 9 hours. Twenty-one hours following the ingestion of milk both animals appeared to be well, had no diarrhoea any longer. The two stools passed before the experiment and the one passed immediately after feeding were normal, no staphylococci could be isolated either from them or from rectal swabs taken at the same points of time, whereas from the stools passed between 4 and 10 hours, from the rectal swabs obtained in the same period and from the 2 vomits innumerable staphylococci were grown. The pathogenic agent was present in small numbers in the stools passed after 24 hours, and not at all in the specimens tested after 48 hours.

Strain II (1.775/61). The two animals fed the infected milk passed 2 stools at two hours, another 2 at five, and 4 at nine hours. From the first four stools innumerable, from the second four somewhat less, *Staphylococcus aureus* could be grown, the rectal swabs taken at 5 hours likewise contained uncountable amounts of the organism. The stools passed before and 30 hours after the experiment were negative for *Staphylococcus aureus*, those passed between 24 and 30 hours contained few of the organism.

Strain III (1.776/61). After a period of latency lasting six hours the two dogs produced one vomit and 5 liquid stools, followed at eight hours by two liquid and at nine hours by 3 semi-solid stools. The vomit and the 7 liquid stools contained large numbers of staphylococcus, whereas the counts were lower in the 9-hour stool and in the further 3 stools passed at night. (The bacterium could be recovered in salt agar only, and not in blood agar.) The 24-hour stools contained staphylococcus in small quantities. The rectal swabs taken after six and eight hours contained enormous quantities of *Staphylococcus aureus*, whereas those obtained at twenty-three hours contained considerably less. After 48 hours staphylococcus could be grown neither from the stools, nor from the rectal swabs. The animals were weak and had no appetite for 5 to 9 hours following feeding.

Strain IV (1.777/61). Between 4 and 8 hours the animals fed the infected milk were weak, had no appetite, passing stools in the following order: Two at 4 hours, one at 5 hours, five at 6 hours; every stool was liquid and contained innumerable staphylococci. After 22 hours five more stools were passed; these were of normal consistency and contained hardly any staphylococcus.

Strain V (1.778/61). Diarrhoea began after 4½ hours and till 9 hours 8 stools were passed. These contained large quantities of staphylococcus. The condition of the animals was similar to that described in connexion with the previous experiments. No *Staphylococcus aureus* could be grown from the stools obtained before, and from those passed later than 30 hours after, feeding. The cultural results of the rectal swabs were similar to those obtained for the stools.

Three dogs were treated rectally with 3 ml volumes of the culture of one of the strains in question. The dogs developed neither diarrhoea, nor any other symptom. The 1—2 stools passed initially and the rectal swabs taken within 6 hours contained small numbers of *Staphylococcus aureus*.

The experiments were repeated with 3 strains of non-haemolytic *Staphylococcus aureus* and with two strains of *Staphylococcus albus*. All these strains originated from food, and gave negative results by the coagulase, lecithinase and mannitol breakdown tests. The animals fed the apathogenic strains showed neither diarrhoea, nor any other symptom following the ingestion of staphylococcus. The bacteria themselves could be demonstrated in the stools for 10 to 24 hours. Similarly, we obtained negative results for the haemolytic *Staphylococcus aureus* isolated from the vomit of a patient with food poisoning, although this strain was coagulase-positive.

In the mouse experiments 24-hour agar cultures of the strains were suspended in saline and 7 to 10 animals were inoculated intracerebrally with a quantity of 0.05 ml. The deaths after 1, 2 and 3 days were recorded. Attempts were made at recovering *Staphylococcus aureus* from the brain and spleen in saline and blood media, and the brain was examined histologically.

Table I

Strain	Number of mice	Number of deaths				Bacteriology result and number			
		total	daily			brain		spleen	
			1	2	3	+	−	+	−
I. 1.774/61	7	7	4	2	1	5	0	5	0
II. 1.775/61	10	10	9	1		7	0	7	0
III. 1.776/61	10	9	8	1		7	0	7	0
IV. 1.777/61	10	9	8	1		7	0	7	0
V. 1.778/61	10	10	9	1		9	0	9	0

The data in Table I indicate that the cultures of the strains originally supposed to be different were practically equal in pathogenicity, the differences obtained could be ascribed to biological scattering. The pathogenic agent could be isolated from the brain and spleen in every case. Pathohistological examination showed oedema and haemorrhage in the brain.

Discussion

In the above described typical but unusually serious staphylococcal food poisoning the source of infection was presumably that member of the family, who had had tonsillitis two weeks earlier, but the other members of the family who had symptoms of angina might have played such a role. Several

foods could be suspected of having had a part in the transmission of the pathogenic agent and the growth of bacteria in them was greatly promoted by the 24 to 48 hours' storage. The vomits and stools of the patients were not examined bacteriologically. These data would have supplemented valuably the record, yet their lack subtracts little of the evidence, it now being unquestionable that staphylococci may be responsible for food poisoning.

The aim of the present investigations was to demonstrate the production of enterotoxin by staphylococci, in the first place. Young cats not having been available, adult dogs were used and it has been ascertained that by the oral administration of milk containing 10^6 to 10^8 live germs per gram of a 24-hour culture of enteropathogenic staphylococci a disease could be induced in them, with a latency of 4 to 5 hours. The most characteristic symptom of the disease was, apart from prostration and anorexia, the frequent passage of mucous diarrhoea, sometimes containing blood, with 4 to 7 stools in 8 to 10 hours. The diarrhoea ceased in 8 to 10 hours, when the other symptoms also disappeared. The pathogenic agent could be demonstrated in large quantities in the first stool samples, later the bacterial count decreased and after 24 hours few staphylococci were present in the stools and rectal swabs. Small numbers of staphylococci may be excreted also under normal conditions. It is to be noted that the isolation of staphylococci, especially when they are present in small numbers only, will only be successful in elective media such as agar containing 8 per cent NaCl, eventually 10 per cent etanol. In blood agar the other members of the normal intestinal flora suppress the staphylococci.

Our results appear to be at variance with those published by MINETT [19]. This is explained partly by our using higher doses than the said author did. We are convinced that the amount of toxin or contaminated food should be proportionate to the weight of the animals. Dogs are undoubtedly less sensitive than are human beings or cats and therefore the dose to be administered should be higher. The symptoms induced are presumably dependent upon the number of bacteria, too, beside the toxin effect.

The results of the intracerebral inoculations of mice indicate that the examined strains of staphylococcus were pathogenic. The differences between strains found in the dog or mouse experiments are due presumably to biological variations. No symptoms could be induced in dogs with an apathogenic strain and with one apparently pathogenic, and therefore we evaluate our results positively. The method we have developed may not be as sensitive as the Dolman test, but still appears to be suitable for the demonstration of enterotoxin production by staphylococci.

We wish to add that on oral administration the staphylococcus produced more serious symptoms in the dogs than the *B. cereus* and other aerobic sporous bacterium strains examined by one of us, and on intracerebral inoculation in mice it was also more pathogenic than *B. cereus* [26, 27].

REFERENCES

1. Feig, M.: Amer. J. Hyg. **40**, 1372 (1950).
2. Bessler, C., Proja, M.: Ann. Sanitá pubbl. **17**, 1195 (1956).
3. Bessler, C., Proja, M.: Ann. Sanitá pubbl. **20**, 955 (1959).
4. Pointeau-Pouliquen, M. A.: Les causes des intoxications alimentaires en France depuis 1920. Libraire Arnette, Paris, 1958.
5. Seeliger, H. P. R.: Bull. Org. mond. Santé **2**, 469 (1960).
6. Csaba, K., Nikodémusz, I., Tarján, R.: Lecture delivered at the Congress of the Hungarian Microbiological Society in 1958.
7. Budapesti Közegészség- és Járványügyi Állomás Évkönyve, 1960.
8. Kuroya, M., Kurose, M.: Tokohu J. Exp. Med. **38**, 599 (1940).
9. Ochring, H.: Z. ges. Hyg. **3/6**, 410 (1957).
10. Gillespie, W. A., Alder, V. G.: J. Path. Bact. **44**, 187 (1952).
11. Krinsky, S., Kedzia, W., Bezla, E., Krasowski, A.: Ann. Inst. Pasteur **102**, 231 (1962).
12. Nestorescu, Popovici, Roman, Voicu, Gutu, Cazareanu: Primul sinposion de Microbiologia alimentelor. Editura Medicala Bucuresti 1959.
13. Dolman, C. E., Wilson, R. J.: Canad. publ. Hlth. J. **19**, 68 (1940).
14. Huet, M.: Arch. Inst. Pasteur Tunis **34**, 473 (1957).
15. Buttiaux, R.: Rev. méd. Liège **11**, 521 (1956).
16. Pisu, I.: Boll. O. M. S. **30**, 1 (1951).
17. Darasse, H.: Ann. Inst. Pasteur **92**, 698 (1957).
18. Kienitz, M.: Zbl. Bakt. I. Abt. Ref. **168**, 123 (1958).
19. Minett, F. C.: J. Hyg. C. (Lond.) **38**, 623 (1938).
20. Ridgon, R. H.: J. Hyg. (Lond.) **72**, 265 (1949).
21. Jordan, E. O.: Proc. Soc. exp. Biol. (N. Y.) **30**, 448 (1933).
22. Sedlák, J.: Z. Immunforsch, **116**, 109 (1958).
23. Kovács, T., Vetró, J.: Népegészségügy **32**, 486 (1951).
24. Pápay, D.: Personal communication.
25. Nikodémusz, I.: 1963 Meeting of the Hungarian Association of Microbiology.
26. Nikodémusz, I.: Zbl. Bakt. I. Abt. Orig.: **184**, 462 (1962).
27. Nikodémusz, I., Gonda, Gy.: Zbl. Bakt. I. Abt. Or. **189**, 298 (1963).

Dr. István Nikodémusz, Budapest, IX. Gyáli út 3/a

Dr. László Kanizsai, Dr. Elek Séllei } Pécs, Bocskai u. 4.

VERGLEICH DER KARIESINTENSITÄT DER ERWACHSENEN BEVÖLKERUNG UNGARNS MIT DEN ANALOGEN ANGABEN DER VEREINIGTEN STAATEN, TSCHECHOSLOVAKEI UND ITALIEN

Von

P. Bruszt

STOMATOLOGISCHE ABTEILUNG DES STÄDTISCHEN KRANKENHAUSES, BAJA

(Eingegangen am 28. November 1962)

Auf Grund von bei annähernd 13 000 Personen über 14 Jahren ohne Selektion durchgeführten Untersuchungen werden die Angaben der Kariesfrequenz und der Kariesintensität bekanntgegeben. Die in 5jährige Altersgruppen geteilten Ergebnisse werden bei Frauen und Männern getrennt bewertet.

Die Kariesangaben der Umgebung der Stadt Baja werden mit den, in den übrigen Gebieten Ungarns gesammelten Daten verglichen.

Die Karieskurven der erwachsenen Bevölkerung von drei anderen Ländern werden mit den Angaben Ungarns verglichen. Während die Kurve der Vereinigten Staaten und die der Tschechoslovakei zueinander recht nahe und über der Kurve Ungarns verlaufen, liegen die Werte Italiens niedriger.

In vorliegender Arbeit wird über die bei nahezu 13 000 Dorfbewohnern (über 14 Jahren) in 12 Gemeinden durchgeführten Untersuchungen berichtet. Die Ergebnisse werden mit einigen zur Verfügung stehenden ausländischen analogen Angaben verglichen. Außerdem werden auch die Ernährungsverhältnisse dieser Länder miteinander verglichen, da im Zustandekommen der Karies unter den Milieuwirkungen annehmbar die Ernährungsweise die wesentlichste Rolle spielt.

Die Untersuchungen fanden in den Jahren 1949—60 im Rahmen von hygienischen Reihenuntersuchungen statt. Über einige Ergebnisse haben wir bereits berichtet [7, 8, 9, 10, 11, 12].

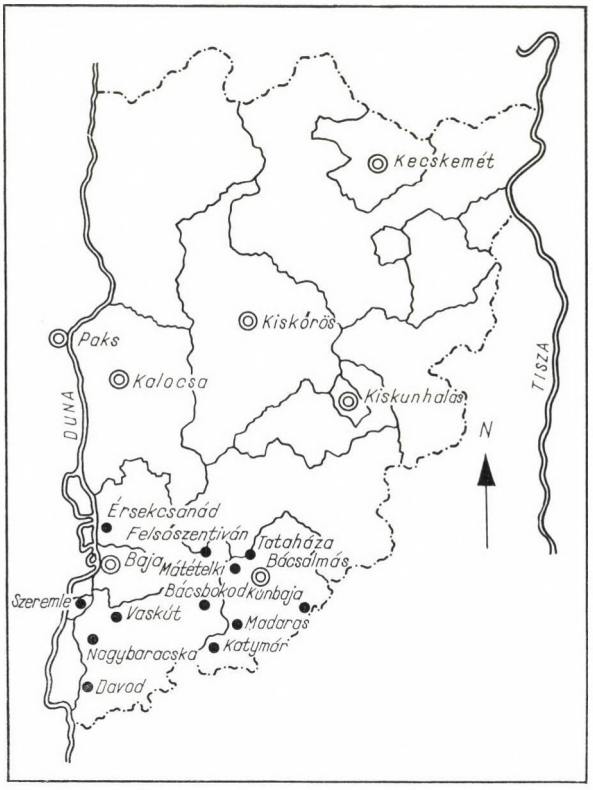
Das untersuchte Gebiet bildet eine geographische Einheit. Asztalos und Sárfalvi [4] schreiben über das Lößfeld von Bácska, wo die untersuchten Dörfer liegen, folgendes:

»Das Lößfeld von Bácska erstreckt sich über die Bezirke Bácsalmás und Baja. Im zwischen der Donau und der Landesgrenze liegenden Gebiet ist — abweichend von seiner Umgebung — die Prozentzahl des Ackerbodens außerordentlich hoch, die Ausdehnung der Wiesen und Weiden dabei unbedeutend. Der Bezirk ist für Zuckerrüben eines der fruchtbarsten. Es werden Getreide, Kartoffeln und außerdem Futterkorn und Halmfutter gebaut. Im Sandboden gedeiht eine hochentwickelte Weinkultur. Hinsichtlich des Viehstandes ist die auf den Maisanbau gegründete Schweinezucht am bedeutendsten.

Das Klima des Gebietes ist kontinental, die jährliche Mitteltemperatur beträgt 10° C, die durchschnittliche Niederschlagsmenge 550—600 mm/Jahr. Die Insolationsdauer, die in Ungarn durchschnittlich 1900 St/Jahr ausmacht, ist am untersuchten Gebiet am höchsten [4, 35].

Mit Ausnahme eines Teiles von Vaskút sind die Bewohner sämtlicher Gemeinden Ungarn [9]. Die überwiegende Mehrzahl der Bevölkerung (71—91,3%, durchschnittlich 81,6%; Angabe aus 1949) sind Feldarbeiter [5]. Sämtliche Länder gehören Kooperativen an.

In den einzelnen Dörfern beträgt die Einwohnerzahl 1080—5982. Durchschnittlich ist pro 100 Wohnungen mit 350 (318—398) Personen zu rechnen [5, 27, 28]. In den Gemeinden gibt es mehrere Bohr- und Oberflächen-



Komitat Bács-Kiskun mit den untersuchten Gemeinden

brunnen. Nur in einem dieser Brunnen (Püspökpuszta bei Dávod) konnte ein hoher Fluorgehalt nachgewiesen werden (3 mg/l), in 5 Brunnen (1 Érsekcsanád, 4 Katymár) betrug der Fluorgehalt etwa 1,5 mg/l, in weiteren 5 dagegen war er außerordentlich niedrig, zumeist unter 0,3—0,4 mg/l.

Die durch das Landesinstitut für Ernährungswissenschaft durchgeführten Untersuchungen beschränkten sich auf die Bewohner der Gemeinden Vaskút und Dávod [9]. Da auf diese Weise nur ein kleiner Teil unseres Materials bearbeitet wurde, stützten wir uns auf die, das ganze Land betreffenden statistischen Angaben [15] (Tab. III).

Es muß betont werden, daß während in den Vorkriegsjahren — das Kindesalter der meisten Untersuchten fällt auf diese Zeit — der jährliche Zuckerverbrauch nur etwa 10 kg betrug [15, 38, 40], diese Zahl heute 17—26 kg ausmacht.

Die Untersuchungsmethodik und die Bearbeitung der Angaben wurden in vorangehenden Mitteilungen beschrieben [7—12].

Insgesamt wurden 12 839 Personen — 6073 Männer und 6766 Frauen — untersucht, also etwa 10% mehr Frauen, als Männer. Diese 12 839 Personen stellen durchschnittlich 43% der erwachsenen Bevölkerung der 12 Gemeinden dar. In 8 von den 14 Altersgruppen erschienen zur Untersuchung mehr als 1000 Personen.

In Tab. I ist die Kariesfrequenz und Intensität der Männer und Frauen in 5jährige Altersgruppen geordnet angeführt. Aus den Angaben geht hervor, daß über 40 Jahren ein kariesfreies Gebiß nur Ausnahmsweise vorkommt.

Die globale *Kariesfrequenz* beträgt bei Frauen 97,7%, bei Männern 95,5%, im Durchschnitt also 96,8%.

Kariesintensität : Der DMF-Index ist bei den 14 Jährigen 3,1, bei den 15—19 Jährigen 4,3 und wächst bei den 20—24 Jährigen von 6,6, 8, 9,4 stufenweise bis zu 19 an. Durchschnittswerte: Frauen 12,5, Männer 10,2, Globalwert 11,4.

Während die Durchschnittswerte (Mittelwerte) — infolge der hohen Zahl der Probanden — nur geringe Fehler aufweisen, zeigen die DMF-Indexwerte in den einzelnen Gruppen beträchtliche Schwankungen und Streuungen (0, 1, 2 . . . 27, 28).

Die 11. Spalte der Tab. I zeigt die zwischen den DMF-Indexen 2 benachbarter Altersgruppen bestehenden Unterschiede. Werden diese Angaben auf 5 Jahre verteilt und die beiden Extremwerte vernachläßigt, so ergibt sich, daß im Durchschnitt jährlich 0,2—0,3 Zähne der Probanden erkranken.

Beachtenswert ist das Übereinstimmen dieser Zahl mit den von ZUHRT [50] jüngstens mitgeteilten Daten über Deutschland.

Mit fortschreitendem Alter ist die Verschlechterung des Gebisses ziemlich gleichmäßig. Das Wort »Zahnverschlechterung« wurde absichtlich gewählt, da unserer Meinung nach im Verderben der Zähne nicht nur der Karies, sondern auch der Lockerung eine Rolle zukommt [43].

In der Kariesbewertung mit Hilfe der DMF-Indexzahl ergibt sich eine große Fehlerquelle aus dem Umstand, daß ein Teil der Zähne nicht wegen Karies, sondern wegen Paradentose fehlt. Das Verhältnis der beiden Ursachen kann nur schwer festgestellt werden. Auf Grund der eigenen Erfahrungen und denen ungarischer Verfasser, ist dem durch Paradentose verursachten Zahnmangel bis zum 40—50. Lebensjahr im allgemeinen keine Bedeutung beizumessen.

Abb. 1 stellt die in den einzelnen Dörfern vorgefundenen DMF-Indexzahlen gesondert dar.

Tabelle I

Kariesfrequenz, Kariesintensität und DMF-Indexkomponente

Alter, Geschlecht	Zahl der Untersuchten	Zahl der Personen mit schlechten Zähnen	%	Zahl der kariösen Zähne	Karies	Wurzel
M.	93	68	73,1	276	1,9	0,2
F. 14	81	68	84,0	273	1,6	0,3
Gesamt	174	136	78,1	549	1,7	0,3
M.	518	438	84,5	2 071	2,1	0,4
F. 15—19	533	472	86,9	2 438	2,8	0,2
Gesamt	1 051	910	86,5	4 509	2,5	0,3
M.	351	332	94,6	1 955	1,9	0,6
F. 20—24	543	499	91,9	3 981	2,5	0,7
Gesamt	894	831	93,0	5 936	2,3	0,7
M.	584	552	94,7	3 878	1,8	0,8
F. 25—29	700	677	96,7	6 492	2,5	0,8
Gesamt	1 284	1 229	95,7	10 370	2,2	0,8
M.	548	523	96,5	4 132	1,6	0,8
F. 30—34	706	700	99,1	7 624	1,9	1,0
Gesamt	1 254	1 223	97,9	11 756	1,8	0,9
M.	622	611	98,2	5 581	1,8	1,3
F. 35—39	634	626	98,7	7 294	1,9	0,9
Gesamt	1 256	1 237	98,3	12 875	1,9	1,1
M.	454	449	99,0	4 453	1,5	1,8
F. 40—44	559	559	100,0	7 343	1,5	1,5
Gesamt	1 013	1 008	99,5	11 796	1,5	1,6
M.	698	677	97,0	7 516	1,2	1,4
F. 45—49	768	764	99,5	10 852	1,6	1,7
Gesamt	1 466	1 441	98,3	18 368	1,4	1,5
M.	650	641	98,6	8 043	1,0	1,8
F. 50—54	725	720	99,3	11 134	1,2	2,0
Gesamt	1 375	1 361	98,9	19 177	1,1	1,9
M.	572	567	99,1	8 261	0,9	1,7
F. 55—59	561	560	99,9	9 451	1,0	1,5
Gesamt	1 133	1 127	99,4	17 712	1,0	1,6
M.	440	436	99,1	6 902	0,8	1,6
F. 60—64	442	442	100,0	8 241	1,0	2,2
Gesamt	882	878	99,5	15 143	0,9	1,9
M.	304	301	99,0	5 102	1,1	2,5
F. 65—69	288	288	100,0	5 519	0,8	1,9
Gesamt	592	589	99,5	10 621	0,9	2,2
M.	157	155	98,7	2 652	0,7	2,6
F. 70—74	166	166	100,0	2 985	0,5	1,9
Gesamt	323	321	99,1	5 637	0,6	2,3
M.	82	82	100,0	1 444	0,5	2,2
F. 75—79	70	70	100,0	1 444	0,8	2,7
Gesamt	152	152	100,0	2 888	0,7	2,5
M	6 073	5 832	95,9	62 266	1,4	1,3
F. 14—79	6 766	6 611	97,7	85 071	1,8	1,3
Gesamt	12 839	12 443	96,8	147 337	1,6	1,3

der erwachsenen Bevölkerung von 12 Gemeinden bei Baja

Füllung	Krone	Zahn-mangel	Karies je Person (DMF-Index)	Unterschied zwischen den DMF-Indexen der 2 Altersgruppen	DMF-Index der Weisheits-zähne	DMF-Index mit den Weisheits-zähnen
0,5	—	0,3	2,9			
1,0	—	0,4	3,3			3,1
0,8	—	0,3	3,1			
				1,2		
0,6	0,1	0,8	4,0			
0,7	0,1	0,7	4,5			4,3
0,6	0,1	0,6	4,3			
				2,3		
1,1	0,3	1,6	5,5		1,2	
0,9	0,6	2,6	7,3		1,5	8,0
1,4	0,4	2,2	6,6		1,4	
				1,4		
0,9	0,4	2,7	6,6		1,3	
1,0	0,9	4,0	9,2		2,0	9,7
1,0	0,6	3,4	8,0		1,7	
				1,4		
0,8	0,5	3,8	7,5		1,5	
1,0	1,2	5,7	10,8		2,1	11,2
0,8	0,7	5,2	9,4		1,8	
				0,8		
1,0	0,8	4,0	8,9		1,8	
1,1	1,1	6,5	11,5		2,3	12,2
1,0	1,0	5,2	10,2		2,0	
				1,4		
0,9	0,6	5,0	9,9		2,2	
0,7	0,7	8,7	13,1		2,5	14,0
0,8	0,7	7,0	11,6		2,4	
				0,9		
0,4	0,4	7,3	10,7		2,3	
0,6	1,1	9,1	14,1		2,3	14,8
0,6	0,8	8,2	12,5		2,3	
				1,4		
0,3	0,3	9,0	12,4		2,5	
0,5	0,8	10,8	15,3		2,8	16,6
0,4	0,6	9,9	13,9		2,7	
				1,8		
0,3	0,3	11,2	14,4		2,8	
0,5	0,7	13,1	16,8		3,0	18,6
0,4	0,5	12,2	15,7		2,9	
				1,4		
0,2	0,2	12,9	15,7		3,1	
0,3	0,4	14,8	18,7		3,2	20,2
0,2	0,3	13,8	17,1		3,1	
				0,8		
0,1	0,2	12,8	16,7		3,0	
0,2	0,4	15,8	19,1		3,4	21,1
0,1	0,3	14,4	17,9		3,2	
				0,1		
0,1	0,5	12,9	16,8		3,2	
	0,1	15,5	18,0		3,4	21,3
0,1	0,3	14,7	18,0		3,3	
				1,0		
	0,1	14,8	17,6		3,2	
	0,1	16,9	20,5		3,5	22,3
	0,1	15,7	19,0		3,3	
0,5	0,4	6,7	10,2		2,2	
0,8	0,8	7,8	12,5		2,4	13,4
0,7	0,6	7,2	11,4		2,3	

Abgesehen von geringzahligen Abweichungen — die vielleicht mit der verhältnismäßig geringeren Zahl dieser Altersgruppen zu erklären sind — liegen sämtliche Werte in der Nähe der Kurve, die die summierten Angaben darstellt und eine nahezu gerade Linie bildet (Abb. 2).

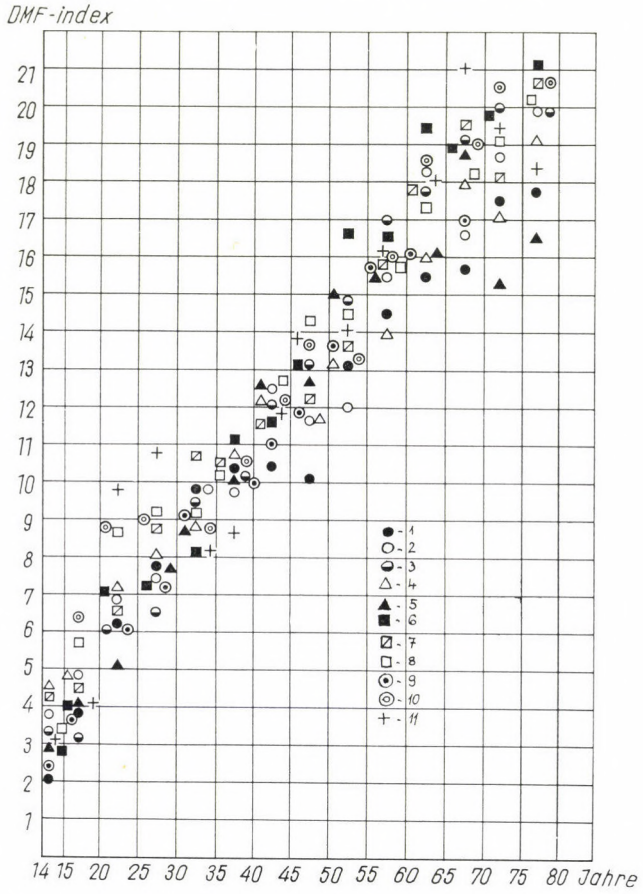


Abb. 1. DMF-Indexe der Bevölkerung von Nagybaracska, Dávod, Vaskút, Bácsbokod, Felsőszentiván, Érsekcsanád, Szeremle, weiterhin von Mátételke-Tataháza, Kunbaja, Katymár und Bácsmadaras, je Altersfünfjahrgruppen. ● 1 Nagybaracska, ○ 2 Dávod, ◒ 3 Vaskút, △ 4 Bácsbokod, ▲ 5 Mátételke-Tataháza, ■ 6 Kunbaja, ▨ 7 Felsőszentiván, □ 8 Érsekcsanád, ⊙ 9 Katymár, ◎ 10 Szeremle ,+ 11 Madaras

Das Durchschnittsalter der Probanden schwankt in den einzelnen Dörfern zwischen 39—43 Jahren, ein 45jähriger Durchschnitt wurde lediglich in einer Gemeinde verzeichnet. Durchschnittswert: 40,8.

Aus den Angaben der Tab. I ist die bereits von anderen Verfassern [3] festgestellte Tatsache ersichtlich, daß die Kariesintensität der Frauen in sämtlichen Altersgruppen schon vor dem Gestationsalter höher ist als die der Männer. Der globale Unterschied zwischen den 2 Durchschnittswerten beträgt 2,3.

Die graphische Darstellung der Komponenten der DMF-Indexe veranschaulicht, daß die Kurve der fehlenden Zähne (wegen Karies und Zahnlockerung verlorene Zähne) und die DMF-Kurve parallel ansteigen (Tab. I). Da bei

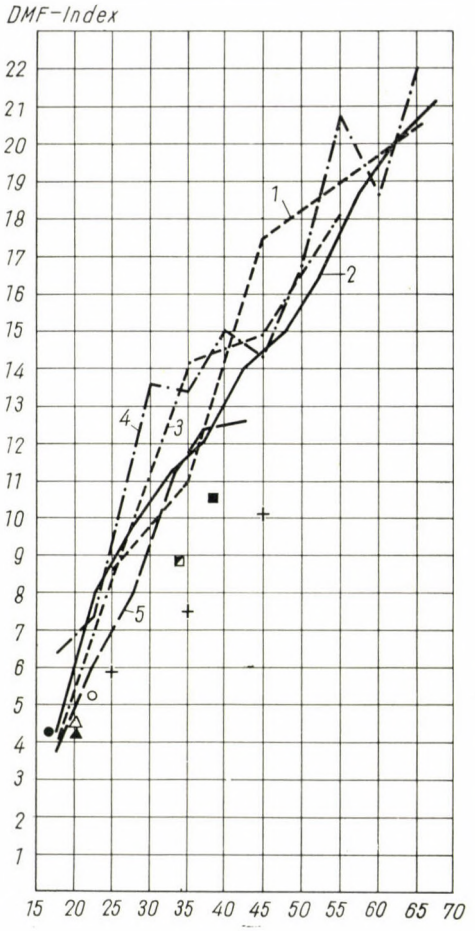


Abb. 2. DMF-Indexe der erwachsenen Bevölkerung in Ungarn (Weisheitszähne einbegriffen). 1. Gyód, 2. Die Bezirke Baja und Bácsalmás, 3. Halas-Majsa, Félegyháza, 4. Kamut, 5. Wöchnerinnen aus Debrecen. ● Mittelschüler aus Budapest. △ Debrecener, Sátoraljaújhelyer, Egerer, ○ Budapester Universitätshörer, ▲ Rekruten aus verschiedenen Gebieten Ungarns. ■ Fabrikarbeiter. ◩ Budapester Blasenmusikanten, + Bergleute aus Rudabánya

der Berechnung der DMF-Indexe einige Verfasser [21, 32, 47 usw.] auch die Weisheitszähne mitrechnen, schien es zweckmäßig, Untersuchungen durchzuführen, inwiefern bei Berücksichtigung der Weisheitszähne in den einzelnen Altersgruppen und global die DMF-Indexe erhöht werden. Auf diese Weise können durch Subtraktion bzw. Addition der die Weisheitszähne betreffenden Daten die Angaben der verschiedene Standpunkte vertretenden Verfasser

verglichen werden. Es ergab sich, daß die Berücksichtigung der Weisheitszähne den DMF-Index der 20—80jährigen um 2,3 erhöht (bei Männern um 2,2, bei Frauen um 2,4). Werden jedoch auch die Altersgruppen zwischen 14—19 in Betracht gezogen, so erhöht die Berücksichtigung der Weisheitszähne den DMF-Index nur um 2,03. Die letzte senkrechte Säule der Tab. I zeigt die Gestaltung der DMF-Indexe nach Addition der Weisheitszähne; die Angaben beziehen sich auf die Altersgruppe über 14 Jahren in den Gemeinden der Umgebung von Baja.

Vergleich der Untersuchungsergebnisse in der Umgebung von Baja und in den übrigen Gebieten Ungarns

Abb. 2 veranschaulicht die in Ungarn gesammelten Angaben über den Kariesbefall der erwachsenen Bevölkerung. Ergebnisse von Ambulanzuntersuchungen, also von Zahnkranken, ferner von Personen deren Trinkwasser hohen Fluorgehalt aufweist bzw. von Probanden, die Berufsschaden ausgesetzt sind, die in einem abgeschlossenem Tal leben, schließlich von Zigeunern, die eine grundsätzlich abweichende Lebensweise führen, werden hierbei nicht berücksichtigt. Aus Abb. 2 läßt sich zwischen den angeführten Angaben — trotz der Fehlerquellen — eine gewisse Gleichmäßigkeit feststellen. Besonders augenfällig ist das Übereinstimmen der Resultate von Gyód, Halas, Majsa, Félegyháza und Kamut mit den Daten, die in der Umgebung von Baja gesammelt wurden. Es soll jedoch betont werden, daß die geringeren Abweichungen der Kariesintensität (DMF-Index) infolge möglicher Fehlerquellen bei der Aufnahme der Angaben nicht als wesentlich zu betrachten sind. Die in mehreren Fällen beobachtete Identität zahlreicher Angaben der Altersgruppe 15—19 ist ebenfalls auffallend.

Laut unserer Angaben bzw. der Abb. 2 ist der Zusammenhang zwischen Lebensalter und DMF-Index in der Altersgruppe 18—80 nahezu geradlinig. Daraus folgt, daß der arithmetische Durchschnittswert der DMF-Indexe in den verschiedenen Altersgruppen ein für das durchschnittliche Lebensalter charakteristischer Wert ist. Diese Feststellung ist auch deswegen von Bedeutung, da teils die Zahl der untersuchten Personen in den übrigen Gebieten Ungarns im Verhältnis zu unserer Probandenzahl nur gering ist (2—300 Personen) [6, 22, 31, 46], teils beziehen sie sich nur auf einige Jahrgänge enthaltende Gruppen [13, 33, 49]. Auf Grund des Gesagten können jedoch auch diese Daten neben Abb. 2 eingezeichnet werden.

Vergleich der Kariesintensität der erwachsenen Bevölkerung Ungarns mit den analogen Daten von drei anderen Ländern

Tab. II und Abb. 3 veranschaulichen, daß die DMF-Indexe Ungarns zwischen den Indexwerten der Vereinigten Staaten und Italiens liegen, d. h. daß der Kariesbefall der ungarischen Bevölkerung mäßiger ist, als der der

Tabelle II

Vergleich der durchschnittlichen Karieszahl je Proband (DMF-Index) in Italien, Ungarn, USA und Tschechoslowakei

(Weisheitszähne einbegriffen)

Altersgruppen	4 Städte in Italien (Schour und Massler)	12 Gemeinden im Komitat Bács-Kiskun (Bruszt)	USA (Klein und Palmer)	Tschechoslovakei (Pončová und Hajek)
11—15	1,5		4,66	6,1
16—20	2,02	4,3	8,50	9,3
21—30	2,31	8,8	17,05	13,36
31—40	6,96	11,7	20,35	17,90
41—50	9,26	14,4	21,87	21,14
51—60	10,80	17,2	23,20	26,07

Vereinigten Staaten, jedoch viel schlechter als bei den Italienern. Erstaunlich ist die Ähnlichkeit zwischen den Kurven der Vereinigten Staaten und der Tschechoslovakei nicht nur in einzelnen, sondern in sämtlichen Altersgruppen, obwohl in der Gestaltung der DMF-Indexe außer Karies und Paradentose auch der zahnärztlichen Versorgung eine Rolle zukommt (die bearbeiteten Angaben stammen aus dem Zeitpunkt vor der Fluoridierung des Trinkwassers).

Bekanntlich werden die kariogenen Faktoren mit der Zivilisation in Zusammenhang gebracht, es ist aber noch nicht endgültig entschieden, welchem Zivilisationsfaktor dabei eine wesentliche bzw. entscheidende Rolle zukommt. Schour und Massler [37] sind der Meinung, daß unter den zahlreichen Faktoren die Ernährungsweise ausschlaggebend ist.

Sós [40] schreibt: »Da Abwechslung und der prozentuale Anteil der Frischprodukte geringer geworden sind, ruft die zivilisierte Ernährung Qualitätsmangelerscheinungen hervor. 1/3 der ärztliche Behandlung beanspruchenden Krankheiten sind Stoffwechselerkrankungen, selbst wenn es zu Beginn irgendeine andere Krankheit war, so wird die zustandegekommene Stoffwechselanomalie das Leitmotiv«.

Dies an sich bietet schon eine Erklärung dafür, daß für die sich mit der Zivilisation parallel verbreitende Zahnkaries die Ernährung verantwortlich ist.

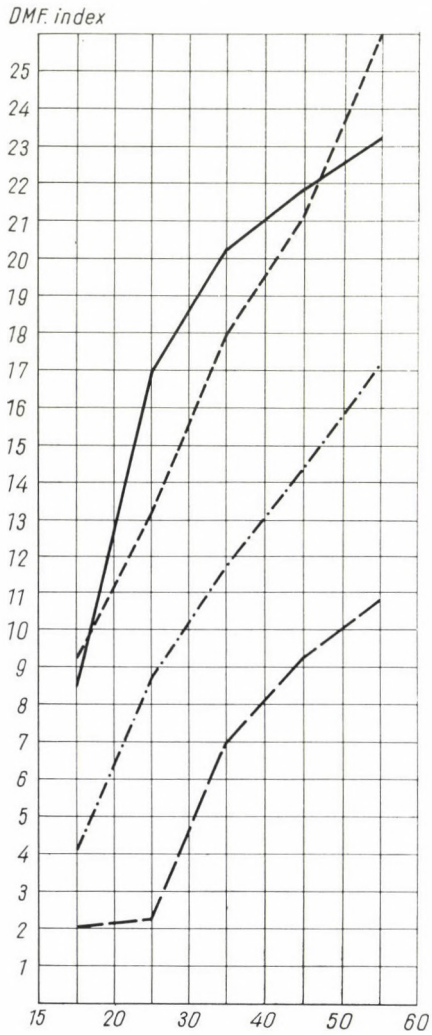


Abb. 3. DMF-Indexe der Bevölkerung über 15 Jahren in der Tschechoslowakei, den Vereinigten Staaten, in Ungarn und Italien

——————— USA
— — — — — — Tschechoslowakei
—.—.—.—.—.— Ungarn
—— —— —— Italien

In Anbetracht, daß die Mehrzahl der Untersuchungen zwischen 1949—60 stattfanden, wurden die Lebensmittelkonsum-Angaben der untersuchten Länder in den Jahren 1955—56 und 1959 verglichen [15, 40]. In Tab. III ist die Gestaltung des jährlichen Lebensmittelkonsums pro Person und der prozentuale Anteil der wichtigsten Lebensmittelgruppen am Kalorienverbrauch zusammengestellt. Laut SCHOUR und MASSLER ist hinsichtlich des Karies-

Tabelle III

Jährlicher durchschnittlicher Lebensmittelverbrauch je Person in den Jahren 1955—56 und 1959

	Menge (kg)				Prozentualer Anteil am Kalorienverbrauch			
	Ungarn	USA	Italien	Tschechoslowakei	Ungarn	USA	Italien	Tschechoslowakei
Getreide	152 *137*	70 *66*	147 *142*	131,5 *125*	48,7 *44,1*	22,6 *21,3*	55,7 *50,6*[a]	44,2 *42,2*
Kartoffel	120 *100*	46 *47*	49 *53*	121,2 *104*	7,4 *6,3*	3,0 *2,9*	3,7 *3,7*[a]	6,8 *6,1*
Zucker	25 *26*	40 *41*	17 *20*	33,7 *36,0*	9,0 *9,7*	15,5 *15,7*	7,1 *7,7*[a]	11,0 *11,7*
Leguminosen und Ölpflanzen	6 *5*	6 *6*	12 *13*	3	2,4 *2,0*	2,5 *2,3*	3,8 *3,6*[a]	
Fleisch	37 *46*	92 *94*	20 *27*	44,8 *54,0*	6,0 *7,9*	16,6 *18,8*	3,7 *4,6*[a]	9,5 *10,5*
Eier	6 *9*	21 *20*	8 *8*	164,0[c] *169,0*[c]	0,7 *1,2*	2,7 *2,5*	1,2 *1,2*[a]	1,2 *1,5*
Fisch	1 *1*	5 *5*	5 *4*	3,7 *4*	0,1 *0,1*	0,6 *0,7*	0,8 *0,7*[a]	
Milch und Milchprodukte[b]	87 *115*	237	106	145,3[d] *111,0*[d]	5,3 *7,3*	14,1 *13,7*	6,3 *6,5*	8,4 *8,7*
Fett und Öl	22 *23*	20 *21*	13 *16*	16,6 *18,0*	16,6 *17,6*	16,1 *16,2*	11,9 *14,2*[a]	13,6 *14,6*

a) Jährlicher Durchschnitt in 1959/60; *b)* Ohne Butter, da Butter in der »Fett«-Säule zu finden ist; *c)* Stück; *d)* Liter.

befalls eher dem Zucker- als dem Zerealienverbrauch eine Bedeutung beizumessen; ich meine weiterhin, daß die zwischen den DMF-Indexen der Vereinigten Staaten und Italiens bzw. Ungarns und der Tschechoslowakei bestehende Unterschiede ebenfalls damit in Zusammenhang stehen.

In den Jahren 1955—59 betrug der Zuckerkonsum in Ungarn 25—26 kg, in den Vereinigten Staaten 40—41 kg, in Italien 17—20 und in der Tschechoslowakei 33—36 kg pro Person. Der Verlauf der Kariesintensitätskurven in diesen Ländern entspricht also annähernd der Reihenfolge des Zuckerverbrauchs. Zum Zeitpunkt der Untersuchungen von SCHOUR und MASSLER war aber der Unterschied zwischen dem Zuckerkonsum der Vereinigten Staaten bzw. Italiens und Ungarns noch bedeutender. (Während der Zuckerkonsum in den Vereinigten Staaten 1930—34 bereits mit dem gegenwärtigen übereinstimmte, betrug die Menge in Italien 9 kg und in Ungarn 10 kg pro Person) [38].

Würde in der Kariesintensität der Getreideverbrauch die Hauptrolle spielen, so müßte die Kariesreihenfolge sich umgekehrt gestalten.

Der Fleisch-, Ei- und Milchkonsum der Vereinigten Staaten ist viel größer als der von Italien bzw. Ungarn. Es scheint jedoch, daß in der Kariesprophylaxe diesen Faktoren keine so wichtige Bedeutung zukommt wie das einige Verfasser annehmen.

Der Anstieg des Zuckerkonsums ist mehr oder weniger gesetzmäßig mit der zunehmenden Industrialisierung verbunden [3]. Nach dem II. Weltkrieg ist in Ungarn die Kariesverbreitung gestiegen, wobei nebst zahlreichen Faktoren auch dem gesteigerten Zucker- und Süßwarenverbrauch eine wichtige Rolle zukommt [3]. Auf Grund der vorliegenden Untersuchungen können wir diesen Umstand hinsichtlich der erwachsenen Bevölkerung mit Daten jedoch nicht unterstützen.

In einer vorangehenden Mitteilung [8] wiese ich darauf hin, daß bei Kindern mit kariesfreiem bzw. verhältnismäßig kariesfreiem Milchgebiß wahrscheinlich auch die bleibenden Zähne gesunder werden. Abb. 3 veranschaulicht, daß wenn in den einzelnen Ländern zwischen den DMF-Indexen in der Pubertät ein stark signifikanter Unterschied besteht: bleibt dieser in sämtlichen Altersgruppen erhalten.

Da die Kurven der einzelnen Länder beträchtliche Unterschiede aufweisen, können die DMF-Indexe Ungarns — trotz der größere Schwankungen aufweisenden Werte einiger Gebiete — als einheitlich betrachtet werden. Dies weist darauf hin, daß im Kariesbefall der Zahnstruktur eine entscheidende Rolle zukommt. Es können aber auch andere Ursachen vorliegen, wie z. B. abweichende Eigenschaften verschiedener Bevölkerungsgruppen, Unterschiede in der Ernährung, ferner sonstige Umweltfaktoren.

LITERATUR

1. Ádler, P.: Fogorv. Szle **48**, 130 (1955).
2. Ádler, P., Bruszt, P., Hradeczky, C.: Dtsch. zahnärztl. Z. **7**, 19 (1952).
3. Ádler, P., Záray, E.: Konzerváló fogászat. II. Auflage. Medicina Budapest, 1961.
4. Asztalos, J., Sárfalvi, B.: A Duna-Tisza köze mezőgazdasági földrajza. Akadémiai Kiadó, Budapest, 1960.
5. Bács-Kiskun megye legfontosabb statisztikai adatai. Kecskemét, 1956.
6. Boros, S.: Fogorv. Szle **44**, 334 (1951).
7. Bruszt, P.: Schweiz. Mschr. Zahnheilk. **67**, 777 (1957).
8. Bruszt, P.: Schweiz. Mschr. Zahnheilk. **68**, 705 (1958).
9. Bruszt, P.: Acta Med. Acad. Sci. hung. **14**, 9 (1959).
10. Bruszt, P.: Népkutató füzetek **1**, 3 (1960).
11. Bruszt, P.: Fogorv. Szle, **54**, 1 (1961).
12. Bruszt, P.: Fogorv. Szle **55**, 102 (1962).
13. Csaba, L.: Fogorv. Szle **47**, 311 (1954).
14. Dunning, J. M.: J. dent. Res. **32**, 811 (1953).
15. Az élelmiszerfogyasztás alakulása Magyarországon. Központi Statisztikai Hivatal, Budapest, 1957.
16. Finn, B. S.: Prevalence of Dental Caries in a Survey of the Literature of Dental Caries. National Acad. Sci. — National Res. Council Washington, 1952.

17. Frisch, J. G.: J. Wis. dent. Soc. **24**, 185 (1948).
18. Hahn, G.: Népegészségügy **30**, No. 9. (1949).
19. Hattyasy, D., Wallner , E.: Fogorv. Szle **44**, 334 (1951).
20. Hattyasy, D.: Népegészségügy **35**, 297 (1954).
21. Holländer, F., Dunning, I. M.: J. dent. Res. **18**, 43 (1939).
22. Huszár, Gy., Göndör, L.: Népegészségügy **29**, 24 (1948).
23. Jackson, D.: Arch. oral Biol. **6**, 80 (1961).
24. Juvancz, I.: Természet és Technika 12 (1949).
25. Klein, H., Palmer, C. E.: On the Epidemiology of Dental Caries. Philadelphia Univ. Press, 1941.
26. Magyarország vármegyéi és városai. Bács-Bodrog vármegye. Monográfia V. Budapest 1909.
27. Magyar Városok és Községek. Statisztikai adatgyüjtemény. Közgazdasági és Jogi Kiadó, Budapest 1958.
28. Magyar Statisztikai Zsebkönyv. Központi Statisztikai Hivatal, **18** (1958).
29. McKay, E. S.: Amer. J. publ. Hlth **38**, 828 (1948).
30. Molnár, L., Sághy, E.: Fogorv. Szle **43**, 379 (1950).
31. Oravecz, P.: Fogorv. Szle **50**, 328 (1957).
32. Pančova, V., Hajek, J.: Čs. Stomat. **60**, 27 (1960).
33. Papp, L.: Vortrag, 1951.
34. Pászner, K.: Népegészségügy **31**, 106 (1950).
35. Radó, L.: Nemzetközi Almanach. Kossuth Kiadó, Budapest, 1959.
36. Schlick, B.: Fogorv. Szle **52**, 178 (1959).
37. Schour, I., Massler, M.: J. Amer. dent. Ass. **35**, 16 (1947).
38. Sós, J.: Magyar néptáplálkozástan. Magyar Orvosi Könyvkiadó, Budapest, 1942.
39. Sós, J.: Gyakorlati eredmények a stomatológiában. 222 o. Bpesti Stom. Klin. Kiadása, Budapest 1947.
40. Sós, J.: Népélelmezés. Medicina, Budapest 1959.
41. Szántó, Gy.: Fogorv. Szle **43**, 282 (1950).
42. Szüle, L.: Népegészségügy **37**, 165 (1956).
43. Szüle, L.: Fogorv. Szle **50**, 50 (1957).
44. Tóth, K.: Fogorv. Szle **46**, 295 (1953).
45. Tóth, K., Mari, L.: Fogorv. Szle **54**, 109 (1961).
46. Tóth, K., Szabó, I.: Fogorv. Szle **54**, 289 (1961).
47. Tóth, K.: Fogorv. Szle **55**, 138 (1962).
48. Várnai, D.: Fogorv. Szle **44**, 335 (1951).
49. Veszprémi, O.: Vortrag 1949.
50. Zuhrt, R.: Dtsch. Stomat. **12**, 475 (1962).
51. 1960. évi népszámlálás adatai. Központi Statisztikai Hivatal, Budapest 1961.

Dr. Pál Bruszt, Baja, Attila u. 6. Ungarn

EFFECT OF MAGNESIUM ON DIETARY INFARCTOID CHANGES IN THE HEART

By

J. Rigó, Gy. Simon, Cs. Hegyvári and J. Sós

with the technical assistance of Maria Schnell and Margit Jóna

INSTITUTE OF PATHOPHYSIOLOGY, UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received December 29, 1962)

In animals fed a magnesium-supplemented, otherwise cardiopathogenic diet no infarctoid changes could be observed in the heart muscle and no decrease occurred in the myocardial potassium and magnesium contents. The electrolyte shifts caused in serum by the cardiopathogenetic diet were also prevented by magnesium.

The potassium content of the perfusion fluid of the Straub heart was significantly lower after 15 minutes, when the magnesium content of the perfusion fluid had been increased to five times the normal concentration.

The myocardial K^{42} content of animals maintained on a magnesium-rich diet was 20 per cent higher than that of the controls 3 hours after intraperitoneal K^{42} administration.

Magnesium deficiency of the diet plays a significant role in the development of experimental cardiopathies. According to Bajusz and Selye [2], diets low in potassium or magnesium make the heart muscle sensitive to pathological changes. Hellerstein et al. [6], MacIntyre and Davidson [8], Unglaub et al. [25], Sós et al. [22, 23] have induced cardiac changes by feeding animals a low-magnesium diet, or low-magnesium diets rich in protein, cholesterol, vitamin D, Ca, PO_4 and Na, and low in K. The results of these investigations called attention to the assumption that magnesium might have a protective action against the development of infarctoid cardiac changes. Selye [17] found $MgCl_2$ to protect against electrolyte steroid cardiopathy. Administration of magnesium alleviated the symptoms even when infarction had been induced by surgical coronary occlusion [2]. Shimamoto et al. [19] reported magnesium to afford protection in myocardial infarction induced by means of macromolecular substances. Since in previous experiments we have found a decrease in the magnesium contents of the entire heart muscle after experimental myocardial infarction [15], it seemed interesting to investigate the prophylactic protective effect of magnesium in the cardiac changes induced by dietary means.

Experimental

Male albino rats weighing 120 to 150 g were used. The control group was fed a semi-synthetic normal diet [21]. Another group was maintained on a

cardiopathogenic diet (S_{65}) [20]. A third group was fed the cardiopathogenic diet, but this time five times the normal magnesium requirement had been added to it, in the form of $MgCl_2$ [16]. After maintaining the animals on these diets for six weeks, they were killed by exsanguination. Part of the heart was studied histologically, the rest was used for electrolyte determinations. Na and K were determined by flame photometry, Ca and Mg by complexometry.

The histological studies indicated that after six weeks on the cardiopathogenic diet 10 animals out of 16 had developed infarctoid cardiac changes, partly myocardial infiltration and partly necroses. In 3 cases severe parenchymatous changes were detected. In 3 animals the myocardium was intact (Table I).

Table I

Effect of cardiopathogenic diet on myocardium

Diet	Condition of myocardium			
	Intact	Mild parenchymatous degeneration	Severe parenchymatous degeneration	Infarctoid change
Cardiopathogenic	3	—	3	10
Cardiopathogenic + magnesium	8	4	—	—
Normal control	10	—	—	—

In response to feeding a cardiopathogenic diet enriched with $MgCl_2$, 8 animals out of 12 showed an intact myocardium. In 4 cases slight parenchymatous degeneration could be demonstrated, although most of the complex noxious factors of the diet (high protein, cholesterol, sodium, phosphate and calcium concentrations, high D_2 vitamin and low K content) had been applied. The results (Table I) make it obvious that the high $MgCl_2$ diet afforded protection against the cardiac changes induced by dietary means.

Since NICKERSON et al. [12] had suggested that an intracellular potassium deficiency would be responsible for the development of infarctoid cardiac changes, whereas DU RUISSEAU and MORI [4] had claimed that a lack of magnesium would be the primary defect, we studied also the changes in the electrolyte content of heart muscle and serum in response to the cardiopathogenic diet and the cardiopathogenic diet enriched with $MgCl_2$. As already reported by us [15], the potassium and magnesium contents of the heart muscle decreased in response to the cardiopathogenic diet. In the present experiments the potassium concentration of the myocardium decreased from 92 mEq/kg to 70 mEq/kg. In the animals fed the magnesium-enriched cardiopathogenic diet no potassium depletion resulted, in spite of the low potassium content of the diet, and no infarctoid changes developed, either.

Similar changes have been noted in the magnesium content of heart muscle. On feeding the cardiopathogenic diet the magnesium content of heart muscle decreased and this change did not take place on feeding a S_{65} diet enriched with magnesium. When magnesium is added to the diet in large amounts, no myocardial calcium and sodium depletion results, either. The results are presented in Table II.

Table II

Changes in myocardial electrolyte concentration

Diet	Number of animals	Potassium	Sodium	Calcium	Magnesium
		mg per 100 g and standard deviation			
Normal	10	370 ± 10	126 ±10	10,5 ± 0.8	20 ± 2
Cardiopathogenic	16	280 ± 15	160 ± 15	14 ± 2	16 ± 2
Cardiopathogenic + magnesium	12	416 ± 35	138 ± 10	11.7 ± 0.5	19 ± 2
		mEq/kg			
Normal		95	55	5.2	16
Cardiopathogenic		72	69	7	13
Cardiopathogenic + magnesium		106	60	5.8	15

The most significant change caused by the cardiopathogenic diet in the electrolyte contents of serum was the decrease of the magnesium level and the increase of calcium level. Neither of these shifts could be observed in the animals fed the magnesium-enriched cardiopathogenic diet. These results are shown in Table III.

Table III

Changes in serum electrolyte concentration

Diet	Number of animals	Potassium	Sodium	Calcium	Magnesium
		mg per 100 ml and standard deviation			
Normal	10	24 ± 3	315 ± 8	9.8 ± 0.1	3.0 ± 0.4
Cardiopathogenic	16	26 ± 2.7	317 ± 12	11.5 ± 0.2	2.2 ± 0.4
Cardiopathogenic + magnesium	12	27 ± 3	312 ± 15	9.4 ± 0.2	3.3 ± 0.2
		mEq/L			
Normal		6.1	137	4.9	2.5
Cardiopathogenic		6.6	138	5.7	1.8
Cardiopathogenic + magnesium		6.9	136	4.7	2.7

In order further to elucidate the phenomenon, we examined the perfusion fluid (Boyle-Conway's solution) of Straub hearts for changes in potassium content at different magnesium concentrations. If the magnesium concentration had been increased fivefold, the potassium content of the perfusion fluid was significantly lower than the control values at 15 minutes. This suggests that in a milieu rich in magnesium the heart muscle might take up increased quantities of potassium. At the end of the experiment no significant difference in the Na content of the Boyle-Conway solution could be demonstrated between the normal hearts and the magnesium-treated ones. These results are shown in Fig. 1.

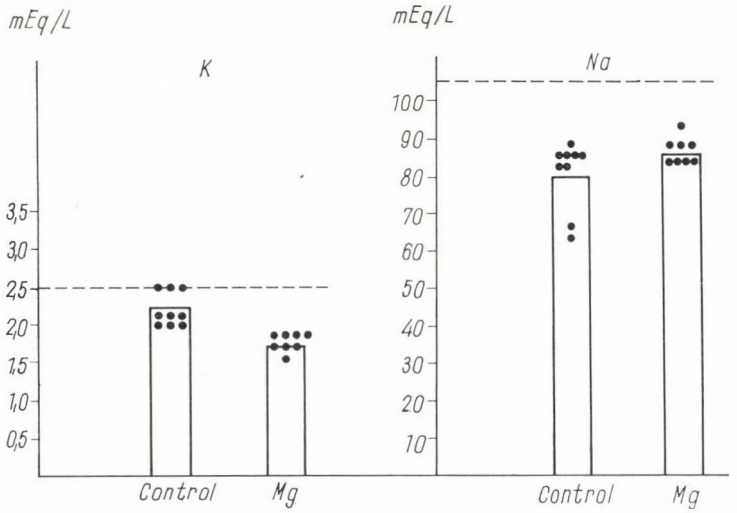


Fig. 1. Potassium and sodium concentration of perfusion fluid 15 minutes after suspension of frog heart. The broken lines indicate the original K and Na concentrations of the Boyle-Conway solution

We have also examined the K^{42} activities of the myocardium of rats fed a normal semisynthetic diet and of rats fed a magnesium-rich diet. The solution containing about 10μC/100 g $K^{42}Cl$ in 0.2 ml isotonic NaCl solution was administered intraperitoneally to a group of 14 animals fed the normal diet and to another group of 14 animals fed the high-magnesium diet. Half of the animals were killed by exsanguination (the carotids were cut) 1 hour, the other half 3 hours, after the administration of the isotope. The heart muscle was homogenised and examined for activity. The results are shown in Fig. 2. No difference in activity could be demonstrated between the two 1-hour groups, whereas the two 3-hour groups differed significantly ($p < 0.05$). The magnesium-rich diet caused a higher K^{42} activity. This indicates that the

presence of large amounts of magnesium reduces the potassium loss by the heart muscle.

These observations suggest a close correlation to exist between magnesium and potassium metabolism.

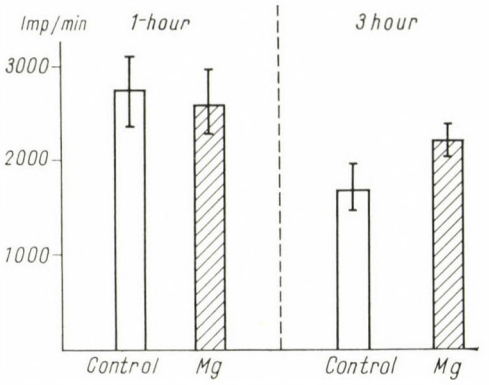


Fig. 2. K^{42} activity of myocardium of animals maintained on normal and on magnesium-rich diets, 1 and 3 hours after intraperitoneal injection of isotope. Ordinate: Impulse/minute/g heart muscle tissue

Discussion

The results obtained with the magnesium-enriched cardiopathogenic diet are in harmony with the effects observed in other types of experimental cardiopathies and also with the favourable effects observed in cases of human myocardial infarction. PEPLIA [14], AGRANAT [1], PARSONS et al. [13] and MARAIS [9] have obtained favourable results in the treatment of angina and myocardial infarction by administering $MgSO_4$ intravenously. KÖHLER [7] observed a beneficial effect of Mg introduced by iontophoresis in the treatment of myocardial infarction. As to the mode of action, it might be suggested that intracellular magnesium plays an important role as a main enzyme activator. According to the observations of HARMOS et al. [5], in response to the cardiopathogenic diet the activity of several myocardial enzymes is decreased. SELYE and BAJUSZ [18] has reported that both KCl and $MgCl_2$ suspend the activity of enzymes causing heart necroses e. g. papaine.

Beside the enzyme activating effect of Mg, the one on mitochondria should also be taken into account. According to MISHRA [10], a magnesium-deficient diet changes the number of cardiac mitochondria. VITALE et al. [26] found that magnesium deficiency produced marked changes in the mitochondria of the heart within a few days. TAPLEY [24] showed that magnesium protects the isolated mitochondria of the rat against various noxious factors causing swelling. According to the investigations of NAKAMURA et al. [11], in response

to feeding a magnesium-deficient diet, mitochondria begin to swell at a time when there is no evidence yet of a lack of magnesium. BARTLEY et al. [3] has assumed that the mitochondrium might be the basic unit responsible for active intracellular electrolyte transport. It is thus possible that the potassium depletion is a result of a deficiency in magnesium.

These experimental results seem to confirm the view that magnesium-rich diets should be used in the prevention of myocardial infarction.

REFERENCES

1. AGRANAT, A. L.: Med. Proc. **4**, 67 (1958).
2. BAJUSZ, E., SELYE, J.: Kísérl. Orvostud. **12**, 283 (1960).
3. BARTLEY, W., DAVIES, R. E., KREBS, H. A.: Proc. Roy. Soc. B. **142**, 187 (1952).
4. DU RUISSEAU, J. P., MORI, K.: Brit. J. exp. Path. **40**, 250 (1959).
5. HARMOS, GY., PUCSOK, J., VARGA, B.: Cardiological Congress, Balatonfüred, Hungary, 1961.
6. HELLERSTEIN, E. E., VITALE, J. J., WHITE, P. L., HEGSTED, D. M., ZAMCHECK, M., NAKAMURA, M., FAHERTY, R.: J. exp. Med. **106**, 767 (1957).
7. KÖHLER, U.: Ärztl. Forsch. **14**, 513 (1960).
8. MCINTYRE, I., DAVIDSON, D.: Biochem. J. **70**, 456 (1958).
9. MARAIS, A. F.: Med. Proc. **4**, 66 (1958).
10. MISHRA, R. K.: Rev. Canad. Biol. **19**, 136 (1960).
11. NAKAMURA, M., NAKATINI, M., KOIKE, M., TORII, S., HIRAMATSU, M.: Proc. Soc. exp. Biol. (N. Y.) **108**, 315 (1961).
12. NICKERSON, M., KARR, G. V., DREZER, P. E.: Circulat. Res. **9**, 209 (1961).
13. PARSONS, R. S., BUTLER, T., SELLERS, E. P.: Med. Proc. **5**, 487 (1959).
14. ПЕПЛИЧ А. Н.: Совет. Мед. **20**, 63 (1956).
15. RIGÓ, K., BUDAVÁRI, I., SÓS, J.: Acta med. Acad. Sci. hung. **17**, 85 (1961).
16. RIGÓ, J., SÓS, J.: Kísérl. Orvostud. In Press.
17. SELYE, H.: The Chemical Prevention of Cardiac Necroses. Reynold Press, New York, 1958.
18. SELYE, H., BAJUSZ, E.: Schweiz. med. Wschr. **88**, 1147 (1958).
19. SHIMAMOTO, T., FUGITA, T., SHIMURA, H., YAMAZAKEN, H., IWAKAWA, S., YAJIMA, G.: Amer. Heart J. **57**, 273 (1959).
20. Sós, J.: Magyar Tud. Akad. Biol. Orv. Tud. Oszt. Közl. **12**, 91 (1961).
21. Sós, J., S. LŐDI, I.: Állatok kísérleti táplálása. In: Kísérleti Orvostudomány Vizsgáló Módszerei, ed. A. G. B. KOVÁCH — Akadémiai Kiadó, Budapest (1957), 1613, p. 230.
22. Sós, J., GÁTI, T., KEMÉNY, T., RIGÓ, J., BUDAVÁRI, I.: Acta med. Acad. Sci. hung. **16**, 190 (1960).
23. Sós, J., GÁTI, T., KEMÉNY, T., RIGÓ, J., BUDAVÁRI, I.: Orv. Hetil. **101**, 1409 (1960).
24. TAPLEY, D. I.: J. biol. Chem. **222**, 325 (1956).
25. UNGLAUB, I., SYLLM-RAPOPORT, I., STRASSBURGER, I.: Virchows Arch. path. Anat. **332**, 122 (1959).
26. VITALE, J. J., NAKAMURA, M., HEGSTED, D. M.: J. biol. Chem. **228**, 573 (1957).

Dr. János RIGÓ
Dr. György SIMON
Dr. Csaba HEGYVÁRI
Dr. József Sós

Orvostudományi Egyetem Kórélettani Intézete, Budapest, IX., Hőgyes Endre u. 9, Hungary

STEROID STUDIES ON FLORISIL ADSORBENT COLUMN

I. BEHAVIOUR OF DIFFERENT STEROIDS ON THE "SERVA" FLORISIL COLUMN

By

I. FAREDIN

FIRST DEPARTMENT OF MEDICINE, UNIVERSITY MEDICAL SCHOOL, SZEGED

(Received January 18, 1963)

Studies of steroid determination with the florisil Serva, Fluka and Nymco, different in adsorptive properties from the florisil extensively used in the literature, have been performed. In the studies with cortisol and cortisone the three makes of florisil purified in the same way as activated under identical conditions, were significantly different in activity.

Further studies have shown that, as applied in chloroformic solution, pregnanediol, allopregnanediol, androsterone, etiocholanolone, isoandrosterone and dehydroisoandrosterone are not adsorbed to Serva florisil, can be easily washed out from it and can be recovered quantitatively.

The results tend to prove that florisil Serva may be used for the separation of cortisol and cortisone, as well as 17-ketosteroids from biological materials.

Author describes a method for the regeneration and activation of different florisils. Using this method the regenerated and activated florisils can be maintained without loss of activity for several weeks.

In view of the great significance of steroid hormones and their metabolites, extensive investigations have been started in recent years to work out methods for the separation and determination of the steroids contained in various biological materials, urine, blood and tissues. It is a complicated task to separate steroids from the contamination of biological materials. Most of the procedures make use of florisil [1, 2], silica gel [3] and Al_2O_3 [4, 5] for that purpose.

Florisil (silicate of magnesium) is known selectively to adsorb corticosteroids; this makes it possible to recover them in pure form from solutions containing different steroids. This useful property of florisil has been utilised by NELSON and SAMUELS [1] in the case of blood and by GLENN and NELSON [2] in the case of urine to separate and identify 17,21-dihydroxy-20-ketosteroid. HERNANDO et al. have further developed the method and used it with success for the separation and identification of urinary aldosterone [6].

Recently, several kinds of florisil ("Serva", West Germany; "Fluka", Switzerland; "Nymco", U. S. A.) have appeared on the market. It seemed interesting to investigate the behaviour of the different florisil preparations against cortisol and cortisone. We have therefore studied the properties of the florisil "Serva", determining first of all how the different steroids (pregnanediol, allopregnanediol, androsterone, etiocholanolone, isoandrosterone, dehydroisoandrosterone) are adsorbed onto, and how they can be removed from, the

column. Then we have investigated whether the "Serva" florisil lends itself for the separation from other steroids of cortisol and cortisone. Finally, a method has been developed for the regeneration of the used florisils and for the preservation of the activity of activated adsorbents.

Experimental procedure

Materials and instruments

1. Washing tube for purification of florisil. This is a glass tube, 10 mm in internal diameter and 10 cm long, with a tap at one end, and a glass vessel of 25 mm internal diameter and about 50 ml in capacity at the other. Do not grease the tap!

2. Florisil chromatography tube, made of Jena glass, with an internal diameter of 6 mm, and a length of 16 cm. There is a glass tap at one end and a glass vessel (internal diameter 20 mm, capacity about 50 ml) at the other. Do not grease the tap!

3. Glass wool. This is boiled in 2 n HNO_3, then in 2 n HCl before use, transferred to a glass filter and washed with distilled water until acid-free, as determined by testing with lithmus paper. Then it is dried in an exsiccator at 120° C for 4 to 5 hours.

4. Absolute ethanol. To the commercial 96 per cent ethanol add 3 to 400 g of heated copper sulphate, allow it to stand 24 hours, shaking it several times. Next day filter and distill under rectifying attachment. To one litre of distilled alcohol add 1 g of m-phenylenediamine 2 HCl, shaking several times. Distill under rectifying attachment and distill the alcohol so purified again, over calcium oxide.

5. Chloroform. Distill over potassium carbonate at 2 to 4-day intervals.

6. 25 per cent ethanolic chloroform.

7. Florisils: 1. "Serva" florisil 100 mesh, Heidelberg, West Germany.

2. "Fluka" florisil 60—100 mesh, Fluka A. G., Switzerland.

3. "Nymco" florisil 60/100 mesh, Floridin Co., USA.

Purification of adsorbents. Fill a small amount of glass wool into washing tube, then add 5 g of commercial florisil. Wash dry column twice with 15 ml absolute ethanol. Dry the ethanolic florisil together with the washing tube in an exsiccator for 6 to 8 hours at 60° to 80° C, then collect the dried, flour-like florisil in a China dish. Then dry the about 100 to 200 g of florisil in an exsiccator at 120° C for 48 hours, to remove traces of alcohol.

Activation of adsorbents. The 100 to 200 g amount of florisil thus pretreated is heated in a furnace at 600° C ± 10° C. If the applied temperature is higher than 600° C, the florisil is so highly activated that the 17-hydroxycorticosteroids cannot be eluted from it.

Regeneration of adsorbents. The 1.5 g florisil column, eluted with 25 per cent ethanol-chloroform in the course of the test, is not discarded, but washed after use twice with 5 ml absolute ethanol, then it is dried together with the chromatography tube for 6 to 8 hours at 60° to 80° C in an exsiccator. The dried florisil is collected, then it is dried in a China dish for 48 hours at 120° C in an exsiccator. The florisil thus regenerated is reactivated as already described.

Preparation of adsorbent column. A small amount of glass cotton is filled into the chromatography tube, then 5 ml chloroform is pipetted into it. In this chloroform are suspended the different florisil preparations in 1.5 g amounts, and are allowed to settle exclusively by gravity. The excess chloroform is allowed to flow out through the tap until its level reaches the surface of the florisil column. The tap is then turned off; the column will attain the desired density.

Purification and activation were the same with every one of the three kinds of florisil.

Procedure

Determined amounts of the ethanolic solution of the steroids to be tested were measured in normal-ground flasks, then were evaporated dry in vacuo at 35° C. To remove traces of alcohol, the steroids were washed twice with

5 ml chloroform in vacuo at 35° C. The steroids thus pretreated were applied with three times 5 ml chloroform to 1.5 g, about 9 to 10 cm high, florisil columns, layer prepared in advance. After the chloroform had dripped down the adsorbent was washed through twice with 10 ml chloroform, then the adsorbed steroids were eluted with 25 ml of 25 per cent ethanolic chloroform. In order to check the adsorptive power of the florisil, the 15 ml chloroform used for the application of steroids, the 20 ml chloroformic washing fluid that had passed through the adsorbent column and the 25 ml eluting fluid were collected in different flasks. In every instance we made a blank test too, by treating a 1.5 g florisil column in the same way.

The solutions thus obtained were evaporated dry in vacuo at 35° C and the amount of the eluted steroids was determined by the corresponding colour reaction.

For the quantitative determination of cortisol and cortisone, the PORTER-SILBER colour reaction [7] was used, with an Optica Milano spectrophotometer type CF 4, at 390, 410 and 430 mμ, in 1 cm quartz cuvettes, on the basis of ALLEN's correction equation [8],

$$E_x = E_{410} - \frac{E_{390} + E_{430}}{2}$$

Pregnanediol and allopregnanediol were determined quantitatively by the colour reaction of KOBER. To the evaporated contents of the flask 5 ml of concentrated sulphuric acid was added, then the lemon-yellow colour that had developed in 30 minutes was photometrized in the spectrophotometer at 400, 425 and 450 mμ, in 1 cm quartz cuvettes. The correction equation of Allen

$$E_x = E_{425} - \frac{E_{400} + E_{450}}{2}$$

was employed in computing the results.

The 17-ketosteroids were determined according to HOLTROFF and KOCH [9], by the Zimmermann reaction, in a Havemann photometer, using 0.5 cm cuvettes and the yellowish-green colour filter.

Results

First, the behaviour of cortisol and cortisone has been studied on florisil columns of different make. 50, 100 and 150 μg amounts of cortisol and cortisone in chloroformic solution were applied to 1.5 g florisil columns purified in the same way and activated under the same conditions, then elution from the columns was examined. The data in Table I reveal that the total amounts of

Table I

Behaviour of cortisol and cortisone on 1.5 g florisil adsorbent columns of different make

Adsorbents tested	Florisil Serva						Florisil Fluka						Florisil Nymco					
Steroid applied μg	Cortisol			Cortisone			Cortisol			Cortisone			Cortisol			Cortisone		
	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150
Steroid found in fluid passed through adsorbent column, μg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Steriod found in washing fluid, μg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Steroid found in eluate, μg	36	77	112	35	71	109	47	94	140	38	75	113	50	99	146	46	85	133
Total steroid recovered, μg .	36	77	112	35	71	109	47	94	140	38	75	113	50	99	146	46	85	133
Percentage recovery	72	77	75	70	71	73	94	94	93	76	75	75	100	99	97	92	85	89

cortisol and cortisone had been adsorbed to every one of the three different florisil columns and were not eluted from them even after washing with chloroform. By means of the eluting solvent, 72 to 77 per cent of cortisol and 70 to 73 per cent of cortisone could be recovered from florisil "Serva". The corresponding values for the florisil "Fluka" were 93 to 94 per cent of cortisol and 75 to 76 per cent of cortisone, for florisil "Nymco", 97 to 100 per cent of cortisol and 85 to 92 per cent of cortisone.

Next, we studied how the different steroids are adsorbed to, and can be eluted from florisil "Serva" 1.5 g columns. The behaviour of pregnanediol and allopregnanediol was examined first. The data in Table II indicate that most of the chloroformic solutions of pregnanediol and allopregnanediol pass through the column together with the solvent, and are completely removed from the adsorbent layer on washing with chloroform, and can be recovered without loss, within the limits of error of the method of determination.

To study the behaviour of a few important 17-ketosteroids, androsterone, etiocholanolone, isoandrosterone and dehydroisoandrosterone were applied to "Serva" florisil columns. The results are shown in Table III. The total amounts of androsterone and etiocholanolone passed through the florisil column without becoming adsorbed, isoandrosterone and dehydroisoandrosterone could be eluted quantitatively from the florisil layer.

Table II

Behaviour of pregnanediol and allopregnanediol on 1.5 g Serva florisil column

Steroid tested	Pregnanediol		Allopregnanediol	
Dose applied, μg	180	370	400	600
Steroid found in fluid passed through adsorbent column, μg	162	320	380	550
Steroid found in washing fluid, μg....	20	50	60	60
Steroid found in eluate, μg	0	0	0	0
Total steroid recovered, μg	182	370	440	610
Percentage recovery	101	100	110	102

Table III

Behaviour of androsterone, etiocholanolone, isoandrosterone and dehydroisoandrosterone on 1.5 g Serva florisil column

Steroid tested	Androsterone		Etiocholanolone		Isoandrosterone		Dehydroiso-androsterone	
Dose applied, μg	200	400	205	411	200	400	200	400
Steroid found in fluid passed through adsorbent column, μg	220	395	200	415	167	370	170	355
Steroid found in washing fluid, μg	0	0	0	0	25	20	22	25
Steroid found in eluate, μg	0	0	0	0	0	0	0	0
Total steroid recovered, μg	220	395	200	415	192	390	192	380
Percentage recovery	110	99	97	101	96	97	96	95

Subsequently, we examined the recovery rates of 50, 100 and 150 μg of cortisol, in the presence of 200 μg of androsterone, isoandrosterone, dehydroisoandrosterone and 400 μg of allopregnanediol. According to the data in Table IV, the quantities of cortisol could be determined with a ±6 per cent error in the presence of these steroids.

Finally, we controlled the activities of the different florisil preparations, regenerated and stored as described, with cortisol. The data of Table V reveal that the extinction values (measured spectrophotometrically by the Porter-Silber colour reaction and corrected according to Allen) of the 50, 100 and 150 μg amounts of cortisol applied to, then eluted from, the different florisils showed no change in 8 weeks.

Table IV

Recovery of cortisol from 1.5 g Serva florisil columns, in the presence of different steroids

Steroids applied	Steroids, μg	Steroids, μg	Steroids, μg
Allopregnanediol	400	400	400
Androsterone	200	200	200
Isoandrosterone	200	200	200
Dehydroisoandrosterone	200	200	200
Cortisol	50	100	150
Cortisol recovered	47	103	155
Percentage recovery of cortisol	94	103	103

Table V

Control of activity of different makes of florisil with cortisol over several weeks

Adsorbents tested	Florisil Serva			Florisil Fluka			Florisil Nymco		
Cortisol applied, μg	50	100	150	50	100	150	50	100	150
Corrected E values of cortisol on freshly activated florisil	0.037	0.080	0.116	0.049	0.097	0.145	0.052	0.103	0.155
One week later	0.037	0.080	0.117	0.049	0.100	0.143	0.053	0.104	0.155
Two weeks later	0.037	0.082	0.115	0.048	0.097	0.142	0.050	0.101	0.158
Four weeks later	0.037	0.080	0.112	0.050	0.098	0.147	0.054	0.104	0.156
Six weeks later	0.038	0.080	0.115	0.049	0.097	0.144	0.051	0.102	0.154
Eight weeks later	0.037	0.081	0.114	0.048	0.098	0.143	0.050	0.103	0.154

Discussion

According to the results obtained, cortisol and cortisone applied in chloroform to the columns, were quantitatively adsorbed to every one of the three different kinds of florisil and could be recovered quantitatively by elution with ethanolic chloroform. The significant differences in recovery rate point to significant differences in quality and activity between the Serva, Fluka and Nymco florisils, purified by the same method and activated under identical conditions.

According to further studies of the recovery of steroids, most of the amounts of pregnanediol and allopregnanediol passed through the Serva column together with the solvent (chloroform) and could completely be removed from the adsorbent by further washing with chloroform, then recovered without loss, within the limits of error, as indicated by the colour reaction. Similar results were obtained for androsterone, etiocholanolone, isoandrosterone and

dehydroisoandrosterone. These results, as well as the data in Table IV suggest that it might be possible to use Serva florisil for the determination of 17-hydroxycorticosteroids in biological materials.

Many methods have been described for the separation of cortisol and cortisone from other steroids and foreign chromogens of biological materials (urine, blood). TALBOT et al. [10] shook over the 17-hydroxycorticosteroids from benzene to the aqueous phase, then extracted them with chloroform. STAUDINGER and SCHMEISSER [11] extracted the steroids from petrolether with 70 per cent methanol. This method became widely employed. However, neither of these procedures proved really adequate, partly because considerable amounts of foreign chromogens were extracted together with the corticosteroids, and partly because there resulted a considerable loss of material. The method based on dialysis developed by BONGIOVANNI et al. [12], as well as the magnesium silicate Celite chromatography of NELSON and SAMUELS [1] tried to eliminate the said sources of error. NELSON and SAMUELS [1] were the first to use florisil for the isolation of 17-hydroxycorticosteroids from blood. Subsequently, GLENN and NELSON [2] applied it with success for the determination of uninary 17-hydroxycorticosteroids, then SANDBERG et al. [13] for the simultaneous determination of 17-ketosteroids and 17-hydroxycorticosteroids eliminated in the urine in glucuronide ester linkages. Since then florisil has been extensively used for the partitioning of steroids in biological materials [6, 14, 15, 16, 17]. The florisil adsorbents applied in those studies were different from those used by us. The authors cited washed with 2 per cent methanol-chloroform the corticosteroids applied with chloroform on the florisil column, then eluted them with 25 per cent methanol-chloroform. In our experiments on washing with 2 per cent methanol-chloroform the total amount of cortisol and cortisone was eluted from every one of the three florisil adsorbents used by us.

The results seem to prove that florisil Serva is suitable for the separation of cortisol and cortisone from other steroids. In possession of this method we may eliminate such procedures of partitioning from the blood and urinary 17-hydroxycorticosteroid and aldosterone assays, as made the methods complicated and often unreliable.

In the light of the above results, florisil chromatography may offer some further possibilities. Cortisone and cortisol being heat-sensitive compounds, their liberation by acid hydrolysis would not produce the desired results, while most of the 17-ketosteroids are present beside glucuronide ester linkage in the form of sulphate ester, and therefore this method would require enzymatic hydrolysis with a combination of sulphatase and beta-glucuronidase.

Our studies concerning the regeneration of florisils and the preservation of their activity have shown that used florisils can be regenerated repeatedly and, if stored as specified, they show no loss of activity in 8 weeks.

REFERENCES

1. Nelson, D. H., Samuels, L. T.: J. clin. Endocr. **12**, 519 (1952).
2. Glenn, E. M., Nelson, D. H.: J. clin. Endocr. a. Metab. **13**, 911 (1953).
3. Nowaczynski, W., Koiw, E., Genest, J.: Canad. J. Biochem. **65**, 639 (1957).
4. Klopper, A., Michie, E. A., Brown, J. B.: J. Endocr. **12**, 209 (1955).
5. Stern, M. I.: J. Endocrinology **16**, 180 (1957).
6. Hernando, L., Crabbé, J., Ross, E. J., Reddy, W. J., Renold, A. E., Nelson, D. H., Thorn, G. W.: Metabolism **6**, 518 (1957).
7. Porter, C. C., Silber, R. H.: J. biol. Chem. **185**, 201 (1950).
8. Allen, W. M.: J. clin. Endocr. **10**, 71 (1950).
9. Holtroff, A. F., Koch, F. C.: J. biol. Chem. **135**, 377 (1940).
10. Talbot, N. B., Salzman, A. H., Wixom, R. L., Wolfe, J. K.: J. biol. Chem. **160**, 535 (1945).
11. Staudinger, Jr., H., Schmeisser, M.: Z. physiol. Chem. **283**, 54 (1948).
12. Bongiovanni, A. M., Eberlein, W. R., Grumbach, M. M., Van Wyk, J. J., Clayton, G.: Proc. Soc. exp. Biol. (N. Y.) **87**, 282 (1954).
13. Sandberg, A. A., Nelson, D. H., Glenn, E. M., Tyler, F. H., Samuels, L. T.: J. clin. Endocr. **13**, 1445 (1953).
14. Gardner, L. I.: J. clin. Endocr. **13**, 941 (1953).
15. Gardner, L. I.: Bull. Johns Hopk. Hosp. **94**, 105 (1954).
16. Herrmann, W., Silverman, L.: Proc. Soc. exp. Biol. (N. Y.) **94**, 426 (1957).
17. Staib, W.: Dtsch. med. Wschr. **87**, 498 (1962).

Dr. Imre Faredin, First Department of Medicine, University Medical School, Szeged, Hungary.

STEROID STUDIES ON FLORISIL ADSORBENT COLUMN

II. A SIMPLIFIED PROCEDURE FOR THE DETERMINATION OF THE TOTAL 17,21-DIHYDROXY-20-KETOSTEROID CONTENT OF THE HUMAN URINE

By

I. FAREDIN

FIRST DEPARTMENT OF MEDICINE, UNIVERSITY MEDICAL SCHOOL, SZEGED

(Received January 18, 1963)

A rapid and reliable procedure for the determination of total 17-hydroxy-corticosteroid content of human urine, suitable for clinical work has been worked out. Instead of spectrophotometry, estimation was carried out by simple photometry. The method described is thought to be suitable for the control of adrenocortical activity in clinical practice. Normal values: for females aged 18 to 50 years, 1.5 to 6.0 mg/24 hours, for males aged 18 to 60, 2.0 to 8.0 mg/24 hours.

According to present knowledge, the only source of 17,21-dihydroxy-20-ketosteroids, or by another term 17-hydroxycorticosteroids, is the adrenal cortex, and for this reason the determination of urinary ketosteroids supplies valuable information as to adrenocortical activity [1, 2]. Several methods have been described for the chemical determination of 17,21-dihydroxy-20-keto-steroids [3, 4, 5, 6]. Owing to its simplicity and sensitivity, PORTER and SILBER'S colour reaction [4] is the one most often employed in clinical practice. With this reaction all the corticosteroids possessing a dihydroxy-acetone side chain on the sterane structure form hydrazone, which gives a greenish-yellow colour with the sulphuric acid-phenylhydrazine reagent. By means of the reaction cortisone, cortisol, 11-deoxycortisol, 6-beta-hydroxy-cortisol [7, 8, 9, 10], as well as the di- and tetrahydrated steroid deriva-tives can be determined together in the urine [11].

Making use of the Porter—Silber colour reaction, many methods have been developed for the assay of urinary 17-hydroxycorticosteriods. In clinical practice the methods of REDDY et al. [12, 13], GLENN and NELSON [14], SILBER and PORTER [15] are widely used.

REDDY et al. [12, 13] extract the free and conjugated 17-hydroxycortico-steroids with n-butanol from urine acidified to pH = 1, and assay it in that extract by a modification of the Porter—Silber colour reaction. Although the method is simple and fast, it is not specific enough. Moreover, n-butanol contains contaminations giving false reactions [13] and a whole series of compounds of non-steroid type [16, 17] make the reliability of the procedure somewhat questionable. For this reason RAFTOPOULO et al. [18], as well as KORNEL [17] and HEBBELYNK [19], have tried to improve the specificity of the method.

At present, the method of GLENN and NELSON [14] is the most widely accepted one. These authors purify the extract obtained by enzymatic hydrolysis of urine on florisil column, first used by NELSON and SAMUELS [20], then apply the Porter—Silber colour reaction and determine the 17-hydroxycorticosteroids by applying ALLEN's correction [21]. Their procedure is specific, sensitive and reliable, but too time-consuming for use in clinical serial studies.

In a previous report [23] we have dealt with the behaviour of different steroids on "Serva" florisil column, different in its properties from the florisils used in earlier works. On the basis of the results obtained we have undertaken, by modifying the method of GLENN and NELSON [14], to develop a simple specific and reliable procedure suitable for serial estimations. We have in addition applied, instead of spectrophotometry, measurement in a Havemann type photometer, in an effort to make the method still easier to carry out.

In the present report we shall describe the method worked out by us and its application for clinical uses.

Experimental

Materials and solutions

1. Chloroform. Distilled over K_2CO_3 at 2 or 3-day intervals.
2. 0.5 mol acetate buffer, pH = 4.5.
3. Penicillin solution. 500,000 U of crystalline penicillin dissolved in 10 ml physiologic saline. Stored in refrigerator.
4. Beta-glucuronidase prepared from the gastric juice of *Helix pomatia* and assayed for activity by a modification of TALALAY's method [24]. In the course of urine hydrolysis, 1000 U/ml enzyme concentration is the one most favourable. The enzyme preparation can be stored for a long time under deep-freeze.
5. Absolute ethanol [23].
6. 1 n NaOH solution (analytical quality).
7. 0.1 n NaOH solution (analytical quality).
8. Na_2SO_4 dry, analytical quality.
9. "Serva" florisil, 100 mesh (Serva, Heidelberg, West Germany). The methods of purification, activation, use and regeneration were the same as described in the previous report [23].
10. Glass wool [23].
11. 25 per cent ethanolic chloroform.
12. Phenylhydrazine, recrystallised three times from absolute ethanol-HCl, then dried over $CaCl_2$ and stored in a dark place in a dark bottle.
13. Diluted sulphuric acid solution, analytical quality. To 190 ml of distilled water are added 310 ml of concentrated H_2SO_4, under constant cooling.
14. Phenylhydrazine-sulphate reagent. 65 ml of phenylhydrazine. HCl are dissolved in 100 ml of dilute sulphuric acid. It may be stored for two weeks in a refrigerator.
15. 10 mg per 100 ml absolute ethanolic solution of cortisol (Organon Oss, Netherlands). To be kept in the refrigerator.

Procedure

Extraction of total 17,21-dihydroxy-20-ketosteroids

To 20 ml aliquot portions of 24-hour collected urine add 7 ml pH = 4.5 acetate buffer. Control pH with indicator paper. Heat content of flask to about 45° C in an about 50° C water bath, then add 25,000 U (0.5 ml) penicillin solution and 20,000 U (0.2 ml) beta-glucuronidase and incubate at 43° for 18 to 20 hours in an incubator.

Cool the incubated urine to room temperature with tap water, then shake out in a 100 ml separatory funnel three times with 15 ml chloroform each, taking care that the chloroform separate well from the urine. Collect extract in a 100 ml ground-stoppered centrifuge tube, then centrifuge for 5 minutes at 300 to 500 r. p. m.

Suck off and discard the urine collected in the upper phase. Add 5 ml of cold 1 *n* NaOH solution to the chloroformic phase, shake vigorously, then centrifuge for 5 minutes. The upper alkaline phase is sucked off and discarded, as before. Repeat this procedure with 5 ml cold 0.1 *n* NaOH solution and 5 ml cold distilled water, as above.

To the chloroformic extract, which may eventually contain also an emulsion layer 2 to 4 mm thick, add 3 g dry Na_2SO_4, shake vigorously, then allow it to stand for 10 minutes. If the Na_2SO_4 has not absorbed all of the water, add further 1 to 2 g of it.

Filter the dehydrated chloroformic extract through cotton into a flask. Wash out the Na_2SO_4 with 5 ml pure chloroform, shaking vigorously, then filter and add the filtrate to the contents of the flask.

Purification of urine extract on florisil column

Apply the dehydrated chloroformic filtrate (about 50 ml) to a previously prepared 1.5 g "Serva" florisil column [23], activated as described. After the chloroformic extract has passed through the column (at a rate of about 1 drop/minute), rinse the flask three times with 15 ml pure chloroform, and wash through the florisil column with this solution. Elute the 17,21-dihydroxy-20-ketosteroids adsorbed onto the florisil with 25 ml 25 per cent ethanol-chloroform into a 100 ml normal-ground flask. Evaporate the eluate under 35° C in vacuum. In the blank test apply 50 ml pure chloroform to another 1.5 g florisil column, then proceed as described above.

Determination of the total urinary 17,21-dihydroxy-20-ketosteroids by the Porter—Silber colour reaction

Dissolve the dry residue in 4 ml absolute ethanol, halve it, then apply the Porter—Silber colour reaction as follows.

a) 2 ml ethanolic extract +5 ml phenylhydrazine sulphate reagent
b) 2 ml ethanolic extract +5 ml dilute H_2SO_4
c) 2 ml absolute ethanol +5 ml phenylhydrazine sulphate reagent

Place each solution into a 60° C (±1° C) water bath for 20 minutes. Subsequently, cool the greenish-yellow solutions with cold water, then subject them to photometry.

Photometry with the spectrophotometer type C. F. 4, Optica Milano

Photometric measurement is carried out at 390, 410 and 430 mμ, in 1 cm quartz cuvettes. At the above wavelengths, using solution *c)*, the spectrophotometer is set to 0, then the extinction values of solutions *a)* and *b)* are read. At the wave lengths the $E_a - E_b$ will be the extinction corresponding to the 17,21-dihydroxy-20-ketosteroids. These values are substituted into the correction equation of ALLEN [21],

$$E_{\text{corr.}} = E_{410} - \frac{E_{390} + E_{430}}{2}$$

and the corrected extinction values thus obtained are read from the calibration curve plotted in the same way by measuring a known quantity of hydrocortisone. This way we get the 17,21-dihydroxy-20-ketosteroids contained in 10 ml urine (before making the colour reaction the ethanolic extract was halved), expressed in cortisol. The cortisol values are computed for 24-hour urine volume and the final result is given in terms of mg/24 hours.

Photometry with Havemann photometer

Photometry was carried out with B. G. 12 mauve filter and 0.5 cm cuvettes. With solution *c* the photometer is set to the 100 scale, then are estimated solutions *a* and *b*. The measured $S_a - S_b$ portion of the scale is read from the calibration curve plotted with cortisol. This gives the amount of 17-hydroxy-corticosteroids in 10 ml urine, expressed in cortisol. The result thus obtained is computed for 24-hour urine output and is given in terms of mg/24 hours cortisol.

Plotting the cortisol calibration curve

Of the 10 mg per 100 ml absolute ethanolic solution of cortisol 0.5, 1.0, 1.5 and 2.0 ml (50, 100, 150 and 200 μg) are pipetted into normal-ground flasks, then the content of every flask is evaporated dry under 35° C in vacuum. The dry cortisol samples are applied in 50 ml chloroform to the previously prepared 1.5 g florisil columns. After the chloroform has dripped down, the adsorbent columns are washed three times with 15 ml chloroform each, then

Cortisol, μg	Spectrophotometer C. F. 4. Optica Milano			Havemann photometer		
	Extinction, corrected ($E_{corr.}$)		Deviation, per cent	Scale reading		Deviation, per cent
	without florisil	with florisil		without florisil	with florisil	
50	0.052	0.038	73	110	82	74
100	0.104	0.079	76	219	163	74
150	0.155	0.112	72	326	238	73
200	0.207	0.155	75	429	322	75

Cortisol calibration curves, plotted with and without florisil treatment, estimated by means of the Optica Milano spectrophotometry and the Havemann photometry

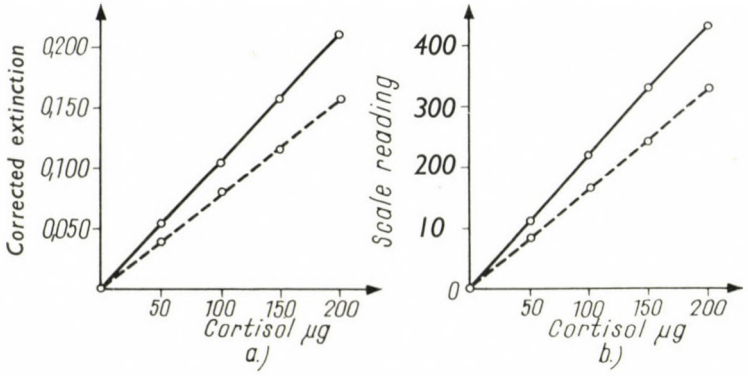


Fig. 1. a) Spectrophotometer C. F. 4. Optica Milano

$$E_{corr.} = E_{410} - \frac{E_{390} + E_{430}}{2}$$

1. ——— without florisil, 2. — — — after florisil; *b)* Photometer Havemann Colour filter B. G. 12. 0.5 cm cuvette 1. ——— without florisil, 2. — — — after florisil

the cortisol is eluted with 25 ml 25 per cent ethanol-chloroform. The eluates are evaporated under 35° C in vacuum, the dry residue is dissolved in 2 ml absolute ethanol and subjected to the Porter—Silber colour reaction. A blank test, too is made. The greenish-yellow solutions thus obtained are subjected to photometry in the spectrophotometer and Havemann photometer, as described above.

It is advisable to control the calibration curves after every florisil activation.

Examinations carried out with the method

In the first part of our methodological investigations, the absorption spectrum of the coloured material resulting from subjecting cortisol to the

Porter—Silber reaction was estimated at 330 to 490 mμ, in the spectrophotometer. After having determined the maximum absorption (410 mμ), we selected the colour filter B. G. 12 (transmittance between 410 and 430 mμ), best suited for work with the Havemann photometer. Then we plotted the cortisol calibration curves obtained without florisilic purification and after purification on the florisil column (Fig. 1). Depending on the duration of activation of the adsorbent and the temperature, 72 to 76 per cent of cortisol could be recovered from the column. Experience has shown that the technique to be adopted is to activate 100 to 200 g amounts of florisil at a time and to plot new calibration curves with every new portion of florisil, as well as to store the florisil as already described [23].

To determine the optimal enzyme concentration, increasing amounts of beta-glucuronidase were added to 20 ml aliquots of 24-hour urines from

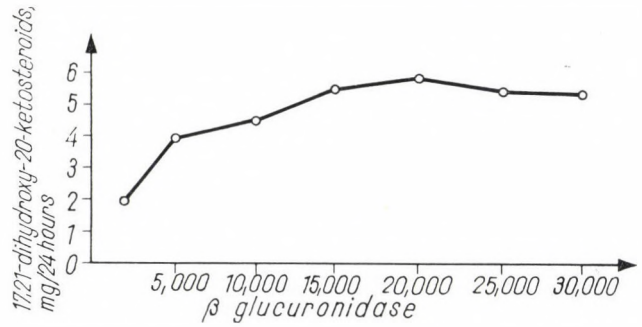


Fig. 2. Release of urinary 17,21-dihydroxy-20-ketosteroids by increasing amounts of beta-glucuronidase, after incubation at 43°C for 20 hours, as determined by the method described, and expressed as mg/24 hours

different patients after the preparation already described. After incubation at 43° C for 20 hours, the urinary hydrolysates were tested for total 17-hydroxy-corticosteroid content. According to our results, 15 to 20 thousand units of beta-glucuronidase are needed to liberate all the 17,21-dihydroxy-20-keto-steroid contained in 20 ml samples of urine (Fig. 2).

Next, we compared the results for total 17,21-dihydroxy-20-ketosteroids in the same urine obtained by spectrophotometry and by measurement in the Havemann photometer. The data in Table I reveal that purification with florisil enhances the specificity of the method to such an extent that there is no substantial difference between the spectrophotometric and the photometric results.

To study the accuracy of the procedure, parallel tests have been done with urines from different individuals. According to the results presented in Table II, the error of 17-hydroxycorticosteroids determination was $\pm$13.6 per cent with spectrophotometry, and $\pm$ 12.3 per cent with photometry.

Table I

Comparison of the results obtained for the Porter—Silber chromogens contained in the same urine extract, estimated by means of the C. F. 4. Optica Milano spectrophotometer and the Havemann photometer

No.	Name	Age, years	Total 17,21-dihydroxy-20-ketosteroids, mg/24 hours		Deviation, per cent
			Spectrophotometer Optica Milano	Photometer, Havemann	
1	T. G.	63	0.65	0.75	—15.4
2	Mrs. P. I.	33	2.12	2.12	0.0
3	Mrs. E. F.	28	2.75	3.16	—14.9
4	Mrs. T. F.	30	3.15	2.94	— 6.7
5	Mrs. G. P.	57	4.54	3.97	—12.5
6	M. A.	16	5.04	4.90	+ 2.8
7	Mrs. P. M.	31	8.43	8.10	+ 3.9
8	Mrs. E. F.	28	10.50	10.20	+ 2.9
9	H. S.	37	13.50	12.40	+ 8.1
10	Mrs. L. A.	26	17.80	18.00	— 1.1
11	Mrs. L. A.	26	21.20	20.90	— 1.4

Table II

Parallel tests for 17,21-dihydroxy-20-ketosteroids in different urines, by spectrophotometry and photometry

Spectrophotometer					Photometer				
No.	Name	Total 17,21-dihydroxy-20-ketosteroids, mg/24 hours		Deviation, per cent	No.	Name	Total 17,21-dihydroxy-20-ketosteroids, mg/24 hours		Deviation, per cent
		A	B				A	B	
1	B. E.	3.85	4.31	—12.0	1	E. I.	2.50	2.50	0.0
2	C. F.	6.66	6.66	0.0	2	C. M.	2.97	2.86	+ 3.7
3	Cs. I.	12.92	11.82	+ 9.3	3	B. E.	4.94	5.52	—11.6
4	L. J.	2.21	2.21	0.0	4	C. F.	7.20	6.93	+ 3.7
5	J. K.	1.46	1.46	0.0	5	K. L.	8.55	8.55	0.0
6	F. B.	3.81	3.30	+13.4	6	S. É.	2.22	2.69	—21.0
7	N. J.	2.02	2.30	— 13.9	7	B. M.	1.24	1.43	—15.3
8	K. M.	2.02	2.23	—10.4	8	L. J.	3.06	2.99	+ 2.3
9	L. I.	3.20	3.42	— 6.9	9	Cs. I.	10.41	10.23	+ 1.7
10	K. M.	3.26	3.48	— 6.7	10	F. B.	3.29	3.29	0.0

For clinical evaluation, urine samples from 20 normal female and 20 normal male patients were tested for total 17,21-dihydroxy-20-ketosteroid content. The result for total 17,21-dihydroxy-20-ketosteroid output was 1.5 to 6.0

mg/24 hours in the case of normal females 18 to 50 years of age, and from 2.0 to 8.0 mg/24 hours in the case of normal males. These normal values are comparable to those reported in recent years as having been obtained by different methods (Table III).

Table III

Normal urinary 17,21-dihydroxy-20-ketosteroid values reported by various authors

Authors	Males			Females		
	Number of cases	Range	Mean	Number of cases	Range	Mean
Reddy et al. [12] 1952	15	2.90 to 12.00	5.80	15	1.10 to 8.60	3.80
Sandberg et al. [26] 1953....	49	3.30 to 9.30	5.30	8	2.10 to 5.80	4.20
Rivoire et al. [27] 1955	15	5.00 to 7.20	6.15	14	4.80 to 6.80	5.82
Marks et al. [28] 1957	42	2.50 to 6.40	4.40	—	—	—
Kornel [17] 1959	10	5.50 to 12.0	8.50	10	3.80 to 9.40	6.40
Faredin [23] 1963	20	2.00 to 8.00	4.13	20	1.50 to 6.00	3.90

To determine how the method lends itself for use in clinical work, we studied the daily total 17-hydroxycorticosteroid output by different patients, before and after treatment with ACTH. The results were in harmony with the clinical picture, and 17-hydroxycorticosteroid output increased following ACTH treatment (Table IV).

Discussion

If carried out as specified, the method described has been found suitable for serial estimations. One trained assistant can make 15 to 20 tests in two days, if proper equipment is available.

We should call attention to some points important for proper evaluation.

According to data in the literature, total 17-hydroxycorticosteroid output depends on the volume of urine excreted. Experience has shown that with a diuresis between 400 and 2000 ml, the 17-hydroxycorticosteroid value is practically constant. Urine can be collected at room temperature, without the use of preservatives, but the samples must be tested within 48 hours. Clinical evaluation should be based on values obtained on two or three consecutive days.

In the course of enzymatic hydrolysis, the reaction of the urine must be between pH 4.5 and 5.2. It is advisable to keep the enzyme concentration at the specified value. If these are not adhered to, the results may be unreliable.

Specificity of the procedure is ensured by washing the urinary extract with alkali (*n* NaOH) and by purification with florisil. If the urinary extract

Table IV

Urinary 17,21-dihydroxy-20-ketosteroids output by different subjects

Name	Sex	Age, years	Diagnosis	Total 17,21-dihydroxy-20-ketosteroid output, mg/24 hours		
				1st day	2nd day	3rd day
N. J.	male	53	Normal	6.88	7.44	—
H. S.	male	55	Normal	6.70	7.14	7.30
J. S.	male	20	Normal	3.32	3.51	3.16
F. I.	male	28	Normal	2.58	2.40	2.67
M. F.	male	52	Normal	3.84	4.12	—
L. K.	female	37	Normal	2.40	2.37	2.75
Cs. I.	female	23	Normal	3.92	4.36	3.96
V. M.	female	27	Normal	5.00	4.26	4.42
B. M.	female	44	Normal	1.60	1.78	1.62
M. G.	female	40	Normal	5.70	5.89	5.40
T. G.	male	36	Hypopituit.	0.48	0.59	0.56
M. M.	male	40	Hypopituit.	0.60	1.16	1.16
Sz. I.	male	13	Hypopituit.	0.28	0.84	0.52
T. E.	female	42	Moderate hypopituit.	1.21	1.60	—
F. J.	female	40	Hypadrenia	1.14	1.70	1.26
B. K.	female	16	Hypadrenia	0	0	0
L. A.	female	40	Cushing's syndrome	22.40	19.08	18.50
M. S.	female	42	Cushing's disease	8.10	7.29	7.01
B. Gy.	female	36	Cushing's disease	17.42	19.98	19.55
J. L.	female	34	Cushing's disease	15.99	13.74	14.08
P. M.	female	30	Cushing's disease	10.30	8.33	10.50
H. E.	female	32	Normal, before treatment	2.20	1.80	2.63
H. E.	female	32	20 U ACTH daily, for three days	3.45	18.04	48.48
P. G.	female	34	Normal, before treatment	3.36	3.73	—
P. G.	female	34	20 U ACTH daily, for three days	9.20	24.40	70.60

is purified inadequately, false Porter—Silber reactions may be obtained after treatment with iodine, KJ, chloralhydrate or paraldehyde [16]. Likewise, if present in the urine, l-ascorbic acid, sugar [17] and lactic acid may interfere with the Porter—Silber reaction. These substances are removed either by alkaline washing, or by the subsequent purification on the florisil column. In the course of washing with alkali, care should be taken that the washing fluids be properly cooled, so as to avoid losses resulting from the water-solubility of the 17-hydroxycorticosteroids. The foreign steroids still present in the urinary extract after washing with base [23] are removed by purification with florisil. Purification with florisil entails a loss of 24 to 28 per cent, due to non-elution from

the florisil column. This is why it is important to observe the specified duration of florisil activation and the specified temperature. With the increase of temperature the adsorptive power of florisil significantly increases [23]. For this reason it is advisable to plot new calibration curves after every florisil activation. If the specifications are adhered to, the method, as indicated by the results in Table I, is reasonably specific for the urinary 17-hydroxycorticosteroids. However, our procedure with spectrophotometric measurement is more reliable.

The 17,21-dihydroxy-20-ketosteroids output values determined by the Porter—Silber colour reaction are given in terms of cortisol units, this being the hormone produced in largest amounts by the normal human adrenal cortex. It is, however, known that not every member of the urinary 17-hydroxycorticosteroids reacts with phenylhydrazine sulphate with the same colour intensity. The most intense colour reaction is given by cortisone; the intensity decreases with cortisol, tetrahydrocortisone and tetrahydrocortisol, in that order [1, 15]. Thus, the quantity of 17,21-dihydroxy-20-ketosteroids determined by the Porter—Silber reaction means not the actually secreted amount, but the quantity of 17-hydroxycorticosteroids related to the colour intensity of cortisol. Hence the term "Porter—Silber chromogens", often used in the literature. Beside the biologically active 17-hydroxycorticosteroids occurring in minute quantities in urine, we determine also the esterified and hydrated steroids. The procedure does not allow to determine the steroids one by one, but, as proved by data in the literature [1, 2] and our own results, it is suitable for the combined determination of the 17-hydroxycorticosteroids produced by the adrenal cortex.

REFERENCES

1. Diczfalusy, E., Birke, G., Plantin, L. O.: Klin. Wschr. **34**, 225 (1956).
2. Loraine, J. A.: The Clinical Application of Hormone Assay, Livingstone, London, 1958.
3. Loewenstein, B. E., Corcoran, A. C., Page, I. H.: Endocrinology **39**, 82 (1946).
4. Porter, C. C., Silber, R. H.: J. biol. Chem. **185**, 201 (1950).
5. Chen, C., Wheeler, J., Tewell, Jr., H. E.: J. lab. clin. Med. **42**, 749 (1953).
6. Appleby, J. I., Gibson, G., Norymbersky, J. K., Stubbs, R. D.: Biochem. J. **60**, 453, 460 (1955).
7. Burstein, S., Dorfman, R. I., Nadel, E. M.: Arch. Biochem. **53**, 307 (1954).
8. Nadel, E. M., Burstein, S., Dorfman, R. I.: Arch. Biochem. **61**, 144 (1956).
9. Frantz, A. G., Katz, F. H., Jailer, J. W.: J. clin. Endocr. **21**, 1290 (1961).
10. Cohn, G. L., Upton, V., Bondy, P. K.: J. clin. Endocr. **21**, 1328 (1961).
11. Staib, W.: Dtsch. med. Wschr. **87**, 498 (1962).
12. Reddy, W. J., Jenkins D., Thorn G.W.: Metabolism **1**, 511 (1952)
13. Reddy, W. J.: Metabolism **3**, 489 (1954)
14. Glenn, E. M., Nelson, D. H.: J. clin. Endocr. **13**, 911 (1953).
15. Silber, R. H., Porter, C. C.: J. biol. Chem. **210**, 923 (1954).
16. Marks, L. J., Leftin, J. H.: J. clin. Endocr. **14**, 1263 (1954).
17. Kornel, L.: Metabolism **8**, 432 (1959).
18. Raftopoulo, R., Staudinger, H. J., Weisbecker, L.: Clin. chim. Acta **4**, 463 (1959).
19. Hebbelynk, M. F.: Clin. chim. Acta **5**, 862 (1960).

20. Nelson, D. H., Samuels, L. T.: J. clin. Endocr. **12**, 519 (1952).
21. Allen, W. M.: J. clin. Endocr. **10**, 71 (1950).
22. Staib, W.: Dtsch. med. Wschr. **87**, 548 (1962).
23. Faredin, I.: Acta med. Acad. Sci. hung. In Press.
24. Talalay, P., Fishman, W. H., Huggins, Ch.: J. biol. Chem. **166**, 757 (1946).
25. Eik-Nes, K.: J. clin. Endocr. **17**, 502 (1957).
26. Sandberg, A., Nelson, D. H., Glenn, E. M., Tyler, F. H., Samuels, L. T.: J. clin. Endocr. **13**, 1445 (1953).
27. Rivoire, R., Rivoire, J., Poujol, J.: J. biol. Chem. **213**, 11 (1955).
28. Marks, L. J., Leftin, J. H., Leonard, M. P.: J. clin. Endocr. **17**, 407 (1957).

Imre Faredin, First Department of Medicine, University Medical School, Szeged. Hungary.

ELEKTROENZEPHALOGRAPHISCHE UND NEUROPSYCHIATRISCHE UNTERSUCHUNGEN BEI HYPERTHYREOTISCHEN KRANKEN

Von

M. Policzer, Erzsébet Moussong-Kovács, Emma Bazsó und M. Marton

INNERE ABTEILUNG (CHEFARZT: DR. M. POLICZER) DES BALASSA KRANKENHAUSES UND PSYCHIATRISCHE KLINIK (DIREKTOR: PROF. DR. J. NYIRŐ) DER MEDIZINISCHEN UNIVERSITÄT, BUDAPEST

(Eingegangen am 1. Februar 1963)

Anhand von 46 Hyperthyreosefällen und 45 an vegetativer Dystonie leidenden Kranken wurde hinsichtlich Beteiligung des Nervensystems, Pathogenese und klinischer Erscheinungsform die Anwendbarkeit der EEG- und der neuropsychiatrischen Untersuchungsverfahren analysiert.

Die Untersuchungen führten zu folgenden Resultaten:

1. Die Zahl der EEG-Anomalien ist bei Hyperthyreose höher, als bei vegetativer Dystonie, gewisse EEG-Erscheinungen gewinnen sogar in stark signifikanter Weise an Übergewicht.

2. Die Zahl der psychischen Noxen emotionalen Charakters erhöhte sich in der hyperthyreotischen Gruppe ebenfalls signifikant.

3. Im Verlauf der EEG-Kurven bzw. in den psychosomatischen Angaben spielen weder die Schwere der Hyperthyreose, noch die Gestaltung der Ergebnisse der durchgeführten Schilddrüsenfunktionsprüfungen eine wesentliche Rolle. Auch den Grundumsatz-Werten über +25% fällt dabei keine Bedeutung zu. Welchen Einfluß der Typ bzw. die klinische Erscheinungsform der Hyperthyreose auf die Untersuchungsergebnisse ausübt, konnte infolge der Zusammensetzung des Materials nicht geklärt werden.

Die mit erforderlicher Kritik bewerteten Ergebnisse der EEG- bzw. neuropsychiatrischen Untersuchungsverfahren können bei der Differentialdiagnostik des »hyperthyreotischen Syndroms« Hilfe leisten.

Der zwischen Hyperthyreose und Funktionsstörungen des Nervensystems bestehende Zusammenhang ist seit langem bekannt [16]. Die ersten Beschreiber des Krankheitsbildes, später aber auch andere Verfasser waren auf Grund der zahlreichen Symptome, die offenbar Folgen der gestörten Nervenfunktion sind — der Meinung, daß der Prozeß, d. h. die Basedowsche Krankheit eine Erkrankung des Nervensystems ist [4, 5, 10, 15]. An Stelle dieser sog. neurogenen Hypothese trat später die von Moebius aufgestellte „thyreogene" Theorie. Obwohl auch die späteren Beobachtungen und Untersuchungen auf die Wichtigkeit des Nervensystems hinwiesen, verlor diese gewissermaßen an Bedeutung, und es entwickelte sich aus den beiden Theorien — nämlich der neurogenen und thyreogenen — eine neue Auffassung. Es gilt heute bereits als anerkannt, daß diese zwei Regulationsmechanismen — der neurogene und der hormonale — als einheitliches System auf die Lebensprozesse bzw. vegetative Erscheinungen funktionell einwirken; das höhere Regulationszentrum dieses Systems ist das unter der Leitung des Kortex stehende Hypothalamus-Hypophysengebiet.

In der Entstehung der, durch die Hyperfunktion der Schilddrüse hervorgerufenen Hyperthyreose bzw. Basedowschen Krankheit kann die Wirkung des Nervensystems auf verschiedene Weisen, d. h. durch verschiedenen Mechanismen zur Geltung kommen.

Der pathologischen Funktion des höheren Nervensystems (Kortex, Hypothalamus) — die entweder die Adenohypophyse oder unmittelbar die Schilddrüse betrifft — fällt im Erkrankungsprozeß unzweifelhaft eine wesentliche Rolle zu [8, 28, 29, 32]. Die Bedeutung der Großhirnrinde bestätigen der auf Wirkung von intensivem, ungewöhnten psychischem Trauma entstehende »Schreckbasedow«, ferner die Experimente von EICKHOFF, der an Hasen mit starken Dauerreizen einen schweren BASEDOW hervorrufen konnte [7]. Diese Auffassung wurde auch von BANSI und Mitarb. [1] unterstützt. Die in organischen zerebralen Prozessen (Encephalitis, CO-Vergiftung, Stromschlag, Hirntraumen) auftretenden hyperthyreoidalen Erkrankungen sprechen für die Rolle des Diencephalons in der Entstehung der Basedowschen Krankheit [34].

Auf Grund des zwischen Hyperthyreose und Nervenfunktionsstörungen bestehenden engen Zusammenhanges konnte angenommen werden, daß die EEG- bzw. die ausführlichen neuropsychiatrischen Untersuchungen zur Klärung einiger Fragen — wie Ursprung und Pathogenese der Hyperthyreose, ferner Form, Typ, Schwere, eventuell Prognose der Krankheit — beitragen würden. Da wir in der Literatur keine diesbezüglichen, ein größeres Material bearbeitenden Mitteilungen fanden, war unsere Zielsetzung die erwähnten Fragen in unserem Krankengut zu analysieren.

Über die Gestaltung der EEG-Kurven bei Hyperthyreose bzw. Basedowschen Krankheit sind in der Literatur mehrere Angaben zu finden.

Früher waren die Forscher der Meinung, daß das EEG-Kennzeichen der Hyperthyreose die diffuse Beschleunigung der Grundtätigkeit ist, die dem bioelektrischen Zeichen der Stoffwechselerhöhung entspricht [12, 26]. THIEBAUT und Mitarb. [30, 31] forschen seit 1948 die elektroenzephalographischen Differenzierungsmöglichkeiten der primären, thyreogenen Form der Hyperthyreose vom sekundären, diencephalischen, hypophysären Typ. Es ergab sich, daß für primäre Hyperthyreose rasche, niedrige alpha-Aktivität mit zahlreichen schnellen Wellenkomponenten (30 Z/sec) charakteristisch ist, während die sekundäre Hyperthyreose durch langsame Wellenserien mit bilateralen, hauptsächlich aber frontotemporalen hohen Amplituden — annehmbar subkortikalen Ursprungs — gekennzeichnet ist; unter Umständen sind außerdem paroxysmale Modulation der alpha-Wellen, sowie ausgeprägte Hyperventilationswirkung nachzuweisen. VAGUE und Mitarb. [33] betonen die Häufigkeit der hohen bogenförmigen beta-Tätigkeit, die sie als die EEG-Manifestation der neurotischen Persönlichkeit betrachten. BECKA und Mitarb. [2] konnten bei Hyperthyreotikern, bei denen die Epilepsie mit Sicherheit auszuschließen war, uni- bzw. bitemporale Spitzenentladungen beobachten. Das Erscheinen

des theta-Rythmus ist laut MILCU und Mitarb. [18] von Bedeutung und weist auf vegetative Gleichgewichtsstörung. Neuestens wird der irritative EEG-Typ — der mit der Vermehrung der Schilddrüsenhormone verbunden ist —, von dem auf TSH-Überschuß weisenden gehemmten EEG-Typ abgesondert [20].

Die beschriebenen Untersuchungen wurden im allgemeinen an geringzahligem Material vorgenommen und ergaben ziemlich abweichende Ergebnisse.

Die neurologische Untersuchung hyperthyreotischer Kranken beschränkt sich meistens auf die klinische Registrierung vegetativer Reize. Anhand eventueller organischer Veränderungen können einige ätiopathogenetische oder lediglich pathoplastische Faktoren (entzündliche, traumatische, toxische oder vaskuläre Nervensystemprozesse) nachgewiesen werden. WOLFSHAUT und Mitarb. [19] sowie MILCU und Mitarb. [19] beschrieben bei Hyperthyreotikern einige auf die Beteiligung der Striopallidum- und Dienzephalonzentren weisende extrapyramidale Symptome (posturale Reflexe, Katalepsie, Gesichtssteifheit).

Auf Grund der sich mit der altbekannten Rolle der emotionellen Faktoren befassenden Forschungen wird angenommen, daß in der Auslösung der Hyperthyreose der individuellen psychodynamischen Struktur der Persönlichkeit eine wesentlichere Bedeutung zukommt, als den das Individuum belastenden Stress-Wirkungen [6, 11, 13].

Krankenmaterial

Die Untersuchungen wurden bei 46 Hyperthyreotikern und bei 45 an vegetativen Regulationsstörungen leidenden Kranken (insgesamt 91 Fälle) durchgeführt. In die erste Gruppe wurden die typischen, mittelschweren und schweren Hyperfunktionsfälle gereiht, bei denen die Diagnose auf Grund des klinischen Bildes, der objektiven Untersuchungen und der Funktionsprüfungen unzweifelhaft Hyperthyreose war. Kriterien der vegetativen Regulationsstörungen waren: 1. Symptome und Beschwerden ohne nachweisbare organische Veränderungen; 2. Beschwerden bzw. Symptome neurogenen Charakters; 3. auch in anderen Gebieten nachweisbare zentrale Regulationsstörungen bzw. vegetative Funktionsstörung. Ein bedeutender Teil der vegetativen Regulationsstörungen entsprach der sog. sympathischen Hypertonie [3], die häufig das klinische Bild einer leichten bzw. mittelschweren Hyperthyreose vortäuscht, die Diagnose der Hyperthyreose aber auf Grund der objektiven Befunde nicht gestellt werden kann.

Die Untersuchung der an vegetativen Regulationsstörungen leidenden Krankengruppe sollte zur Klärung folgender Fragen beitragen:

1. Soll der zwischen neuropsychiatrischen Faktoren und EEG-Veränderungen bestehende Zusammenhang als »spezifisch« betrachtet werden, oder

kommen diese Erscheinungen auch bei Nervensystemfunktionsstörungen anderen Ursprungs vor?

2. Besteht eine Möglichkeit, daß sich aus einem Teil der vegetativen Regulationsstörungen mit der Zeit, auf Einwirkung verschiedener Faktoren, durch die einzelnen Stadien des Präbasedows typische Hyperthyreose bzw. Basedowsche Krankheit entwickelt? [23].

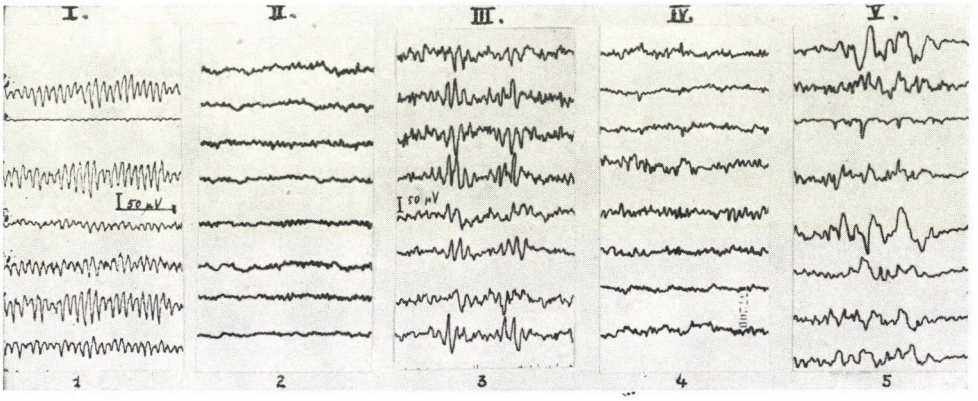


Abb. 1 a. EEG-Typen bei Hyperthyreose: I.: Normaler alpha-Rhythmus; II.: Beschleunigte Grundtätigkeit (beta-Rhythmus). III.: Paroxismal modulierter alpha-Rhythmus. IV.: Diffuse Dysrhythmie. V.: Paroxysmale Dysrhythmie subkortikalen Typs

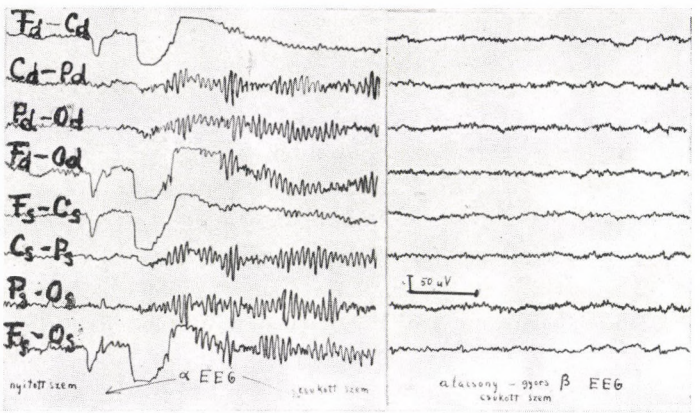


Abb. 1 b. Normaler alpha-Rhythmus bei Hyperthyreose l. s. (I.). Schneller beta-Rhythmus l. d. (II.)

Methodik

Außer den üblichen Untersuchungen wurden in sämtlichen Fällen folgende Schilddrüsenfunktionsprüfungen durchgeführt: J^{131}-Speicherungskurve der Schilddrüse, Plasmatest, Plasmaaktivität nach 2, 48 Stunden, Harnaktivität in 3 Fraktionen (0—8, 8—24 und 24—48 Stunden), Jodgehalt des Serumeiweißes, Grundumsatz (Krogh'sche Methodik bzw. Diapherometer), Serum-Cholesterin-Gehalt. Die Methodik der Untersuchungen wurde in einer vorangehenden Mitteilung bereits bekanntgegeben [25].

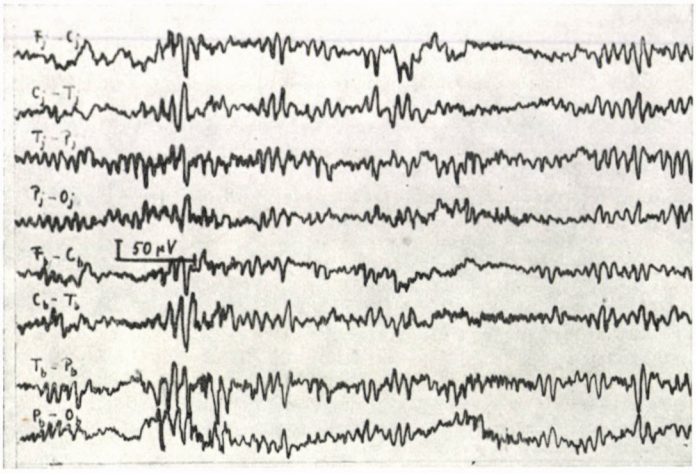


Abb. 2. Paroxysmal modulierter alpha-Rhythmus bei Hyperthyreose (III.)

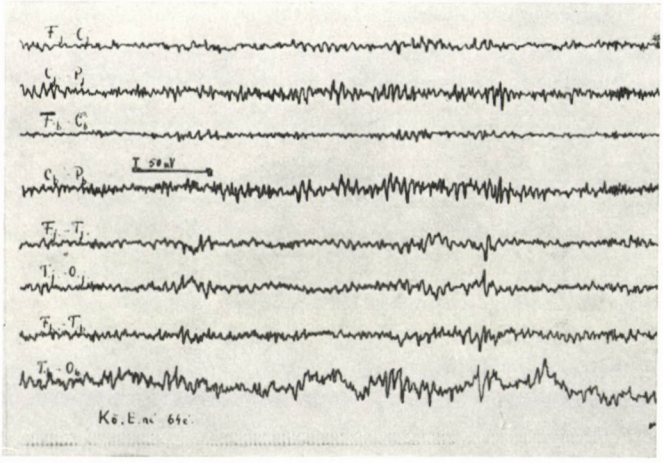


Abb. 3. Diffus dysrhythmischer Rhythmus mit temporalen Spitzen bei Hyperthyreose (IV.)

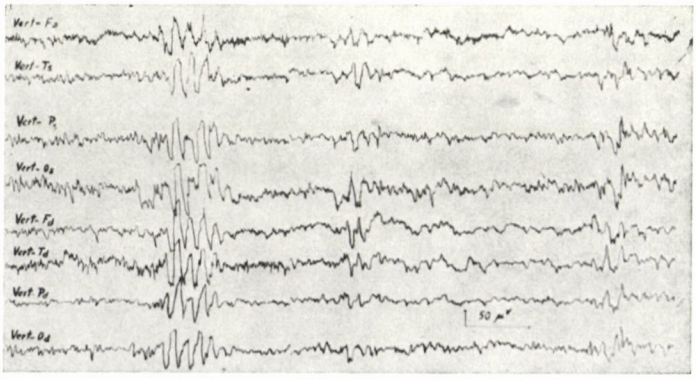


Abb. 4. Paroxysmale Dysrhythmie subkortikalen Typs bei Hyperthyreose (V.)

In sämtlichen Fällen wurde ein sog. vegetativer Fragebogen ausgefüllt, bestimmte anamnestische Angaben kontrolliert und die verordneten Untersuchungen durchgeführt [22]. Hinsichtlich der somatischen Anamnese wurden folgenden Standpunkten besondere Aufmerksamkeit gewidmet: eventuelle Enzephalitis oder Meningitis (som. 1.), Epilepsie (som. 2.), Kommotion (som. 3.), schwere asthenisierende Erkrankungen, physiche Erschöpfung (som. 4.). Die bei der Entstehung der Hyperthyreose eventuell bestehende kritische endokrine Periode (Entbindung, Klimax) (som. 5.) sowie sich zur Hyperthyreose gesellende kortikoviszerale Erkrankungen (Hypertonie, Ulkus, usw.) (som. 6.) wurden ebenfalls berücksichtigt.

Das bei den Kranken vorgenommene »psychiatrische Interview« trug zur Klärung folgender Fragen bei: Bestehung und Form einer eventuell neurotischen Grundpersönlichkeit (psych. 1.), im Kindesalter erlittene emotionelle Belastungen (psych. 2.), einmalige intensive Schockwirkung (psych. 3.), anhaltende psychische Spannung bzw. häufige kleine Psychotraumen (psych. 4.), außerdem wurde auch die Tatsache eines neuen geistigen Arbeitskreises berücksichtigt (psych. 5.).

Die EEG-Aufnahmen wurden mit einem KAYSERschen, 8 Kanal-Tintenschreiberapparat verfertigt: Hyperventillations- und intermittierende Lichtreizbelastungen wurden in sämtlichen Fällen vorgenommen. Da das Ziel vorliegender Arbeit teils auch die Nachuntersuchung der bislang mitgeteilten Literaturangaben war, wurde bei der Gruppierung der EEG-Befunde die neuere, 1961 festgestellte Nomenklatur nicht angewendet. Die EEG-Aufnahmen wurden in 5 Gruppen gereiht:

I. Normaler alpha-Rhythmus (bis 9—11 Z/sec).
II. Beschleunigte Grundtätigkeit (alpha- und beta-Rhythmus: 12 und 13 Z/sec)
III. Paroxysmaler modulierter bzw. hypersynchroner alpha-Rhythmus.
IV. Diffuse Dysrythmie (Frequenz-Labilität und eventuelle temporale Spitzen inbegriffen).
V. Paroxysmale Dysrhythmie subkortikalen Typs (Abb. 1).

Die statistische Bewertung der Ergebnisse wurde mit dem x Quadrat-Verfahren durchgeführt.

Ergebnisse

Tab. 1. veranschaulicht Alter, Geschlecht und Beschäftigung der Patienten.

Die den Krankheitsgruppen entsprechende Verteilung der EEG-Befunde ist in Tab. II. dargestellt.

Die angeführten Angaben führen zu folgenden Feststellungen:

Tabelle I

		Hyperthyreose	Vegetative Regulationsstörung
Geschlechts-verteilung	Männer	11	15
	Frauen	35	30
Lebensalter		46,7 Jahre (20—72 Jahre)	39,4 Jahre (12—67 Jahre)
Berufsverteilung	Intellektuellen	15	22
	Physische Arbeiter	25	20
	Gemischt	6	3

Tabelle II

Krankheitsgruppen	EEG-Gruppen				
	I.	II.	III.	IV.	V.
Hyperthyreose (46 Fälle)	4	8	7	7	20
Vegetative Regulationsstörungen (45 Fälle)	21	4	6	6	8

1. *Milde oder ausdrückliche EEG-Anomalien* (Gruppe II—III—IV—V):

Bei Hyperthyreose in 42 von 46 Fällen (91%), bei vegetativen Regulationsstörungen in 24 von 45 Fällen (53%). Die Differenz ist stark signifikant ($p < 0{,}1\%$).

2. *Für Hyperthyreose charakteristisch betrachtete EEG-Anomalien* (Gruppe II—III—V):

Bei Hyperthyreose in 35 von 46 Fällen (76%), bei vegetativen Regulationsstörungen in 18 von 45 Fällen (40%). Die Differenz ist stark signifikant ($p < 0{,}1\%$).

3. *Verhältnis der normalen und schweren EEG-Anomalien :*

Bei Hyperthyreose (Gruppe I—V) in 4 von 20 Fällen, bei vegetativen Regulationsstörungen in 8 von 21 Fällen. Die Differenz ist stark signifikant ($p < 0{,}1\%$).

4. *Als Zeichen der primären Schilddrüsenerkrankung betrachtete EEG-Anomalien* (Gruppe II):

Bei Hyperthyreose in 8 von 46 Fällen, bei vegetativen Regulationsstörungen in 4 von 45 Fällen.

Der sekundären Hyperthyreose zugeschriebene subkortikale EEG-Zeichen :

Bei Hyperthyreose in 27 von 46 Fällen, bei vegetativen Regulationsstörungen in 14 von 45 Fällen.

Aus Tab. III ist die Verteilung der somatischen und psychischen Faktoren in 53 Hyperthyreose- und 38 vegetativen Regulationsstörungsfällen ersichtlich (Bezeichnungen s. Methodik).

Auf Grund der Daten der Tabelle III sind folgende Zusammenhänge festzustellen:

1. *Die summierten som. 1., 2., 3. Faktoren* kommen bei Hyperthyreose in 53 Fällen 6mal, bei vegetativen Regulationsstörungen in 38 Fällen 11mal vor. Bei vegetativen Regulationsstörungen ist also — im Gegensatz zu den Hyperthyreose-Fällen — das Übergewicht der das Nervensystem unmittelbar betreffenden Noxen zu beobachten.

Tabelle III

Krankheitsgruppen	Psychosomatische Faktoren										
	Somatische Faktoren						Psychische Faktoren				
	1.	2.	3.	4.	5.	6.	1.	2.	3.	4.	5.
Hyperthyreose (53 Fälle)	2		4	17	16	7	16	4	28	23	2
Vegetative Regulationsstörungen (38 Fälle)	3	6	2	13	10	5	10	6	17	5	1
	Dieselben Faktoren in der V. EEG-Gruppe										
Hyperthyreose (21 Fälle)	1		1	9	5	1	8	2	12	13	1
Vegetative Regulationsstörungen (7 Fälle)		4		2	3	1	2	2	2	1	

2. Am verhältnismäßig häufigsten kommen die somatischen Faktoren der Gruppe 4 und 5 vor, ihre Verteilung ist jedoch der Zahl der Fälle proportional:

Bei Hyperthyreose in 33 von 53 Fällen, bei vegetativen Regulationsstörungen in 23 von 38 Fällen.

3. Obwohl die Prozentzahl der neurotischen Persönlichkeit (psych. Gruppe 1) unter den psychischen Faktoren recht hoch ist, kann bei den 2 Gruppen eine proportionale Verteilung festgestellt werden:

Bei Hyperthyreose in 16 von 53 Fällen, bei vegetativen Regulationsstörungen in 10 von 38 Fällen.

4. Vorkommen der psychischen Faktoren der Gruppe 3 und 4 (einmalige intensive Schockwirkung und dauernde emotionale Spannung):

Bei Hyperthyreose in 51 von 53 Fällen, bei vegetativen Regulationsstörungen in 22 von 38 Fällen. Die Differenz ist stark signifikant ($p < 0{,}1\%$).

5. Wird die Verteilung der gesamten somatischen und psychischen Faktoren in beiden klinischen Gruppen geprüft, so ergibt sich, daß während diese bei vegetativen Regulationsstörungen je 50% beträgt (39—39), kommen bei den Hyperthyreose-Kranken die psychischen Einwirkungen zweimal häufiger vor als die somatischen Noxen (43—73).

6. Der untere Teil der Tab. III veranschaulicht die Angaben der oben angeführten Zusammenhänge hinsichtlich der schwereren EEG-Anomalien (Gruppe V). Die Verteilung sämtlicher schädigender Faktoren (somatische + psychische) gestaltet sich in den einzelnen Gruppen der Zahl der Fälle proportional (53 : 21 bzw. 17 : 7). Während jedoch die summierten somatischen Faktoren der Gruppen 1, 2, und 3 bei den vegetativen Regulationsstörungen an Übergewicht gewinnen (2 : 4), entspricht die Verteilung der Faktoren der Gruppe 4 und 5 der Zahl der Fälle (14 : 5).

Die summierten psychischen Faktoren der Gruppe 1, 3 und 4 kamen: bei Hyperthyreose in 33 von 53 Fällen, bei vegetativen Regulationsstörungen in 5 von 38 Fällen vor. Die Differenz ist signifikant (p zwischen 1 und 2%).

7. Das gemeinsame Vorkommen von 3 oder mehreren Faktoren bei demselben Patient:

Bei Hyperthyreose in 20 von 53 Fällen, bei vegetativen Regulationsstörungen in 11 von 38 Fällen. Die Differenz ist nicht signifikant ($p > 30\%$).

Auf Grund der Ergebnisse der EEG- und neuropsychiatrischen Untersuchungen kann zusammenfassend festgestellt werden, daß — obwohl bei vegetativen Regulationsstörungen als subjektive und objektive Symptome der Regulationsstörung eine größere Anzahl von neurotischen Erscheinungen und EEG-Anomalien zu erwarten war — zwischen den beiden Gruppen sich meistens entgegengesetzte, stark signifikante Unterschiede manifestierten. In der hyperthyreotischen Gruppe war nicht nur die Zahl sämtlicher EEG-Anomalien höher, als in der Gruppe der vegetativen Regulationsstörungen, sondern auch die für Hyperthyreose charakteristisch betrachtete EEG-Erscheinungen gewannen in stark signifikanter Weise an Übergewicht.

Während in der Gruppe mit vegetativen Regulationsstörungen die somatischen und psychischen schädigenden Faktoren im gleichen Verhältnis vorkamen, war in der Hyperthyreose-Gruppe (und zwar am ausdrücklichsten in der mit schweren subkortikalen EEG-Anomalien verlaufenden Gruppe V) das signifikante Übergewicht der psychischen Noxen emotionalen Charakters zu beobachten. In der Bewertung der Ergebnisse muß unbedingt in Betracht gezogen werden, daß die bei der hyperthyreotischen Gruppe beobachteten EEG-neuropsychischen Abweichungen nicht mit gesunden, über normale Regulation verfügenden Personen verglichen wurden, sondern mit den Ergebnissen der an vegetativer Regulationsstörung leidenden Patienten. Mit Hilfe dieser Methodik können die bei Hyperthyreose gefundenen Veränderungen mit den von der Hyperthyreose unabhängigen, durch verschiedenen Mechanismen entstandenen Nervensystemfunktionsstörungen verschiedener Ätiologie verglichen bzw. gesondert bewertet werden.

Tab. IV veranschaulicht, welcher der in der ersten senkrechten Spalte angeführten Faktoren in der Gestaltung der EEG- bzw. neuropsychischen Untersuchungsergebnisse die Hauptrolle spielt. Das Material wurde folgenden Gesichtspunkten entsprechend eingeteilt:

1. Schwere der Hyperthyreose auf Grund objektiver Untersuchungsergebnisse (6, 5, 4, 3 positive Resultate von den durchgeführten 6 Bestimmungen).
2. Grundumsatzwerte über +25%.
3. Hyperthyreose-Typen:
   a) dienzephalische, hypophysäre, primäre Hyperthyreose

Tabelle IV

Neurologische Untersuchungen. Klassifizierung der Hyperthyreose		EEG-Befunde					
		Gesamtzahl	I.	II.	III.	IV.	V.
Positive Hyperthyreose-Funktionsproben Anzahl	6 + 5	19	2	4	3	2	8
	4 + 3	16	2	3	1	2	8
	Grundumsatz > + 25%	33	4	6	6	3	14
	Plasmaproteinjod > 7,5 μg%	29	3	7	2	4	13
	J^{131}-Aufnahme > 31%	35	3	7	7	3	15
	J^{131}-Speicherung > 56%	32	2	7	6	3	14
	Plasmatest > 1,0	31	4	3	6	5	13
	Se-Cholesterin > 140 mg%	15		2	2	3	8
Struma	Diffusa	39	3	6	5	6	19
	Nodosa	5		2	2		1
	Exophthalmus	38	4	8	3	6	17
	Kein Exophthalmus	7			4		3
Typ	Dienzephalischer	29	3	4	5	5	12
	Hypophysärer	14	1	4	1	1	7
	Primärer	4		1			1

b) Fälle mit bzw. ohne Exophthalmus

c) diffuse Struma bzw. Knotenkropf.

Aus Tab. IV ist ersichtlich, daß die EEG- bzw. neuropsychischen Veränderungen mit keinem der in der ersten senkrechten Säule angeführten Angaben parallel verlaufen. Es kann vielmehr festgestellt werden, daß die gewonnenen Resultate übereinstimmend sind: Die meisten Fälle gehören — und dies gilt für sämtliche Einteilungen — in die EEG-V bzw. in die auf Hyperthyreose charakteristischen II—III—V-Gruppen. Im Gegensatz zu den Hypothesen mehrerer Verfasser werden auf Grund der Resultate sämtlicher Schilddrüsenfunktionsprüfungen und der Erhöhung des Grundumsatzes ähnliche Ergebnisse erhalten. Die besondere Rolle des Grundumsatzes wird selbst von der EEG-Verteilung unserer 12 Fälle (aus dem Material mit vegetativen Regulationsstörungen), deren Grundumsatzwerte über +25% waren, nicht unterstützt: In der Gruppe I, sechs, in den Gruppen II—III—V, je zwei. In dieser

Somatische Faktoren							Psychische Faktoren					
Gesamtzahl	1.	2.	3.	4.	5.	6.	Gesamtzahl	1.	2.	3.	4.	5.
15	2		3	6	3	1	21	4	2	9	6	
19			1	10	6	2	26	6		9	8	3
30	2		2	15	7	4	49	12	4	18	13	2
30	1		3	14	9	3	34	6	1	15	10	2
26	2		4	8	10	2	44	10	3	18	11	2
24	2		4	6	10	2	41	10	2	17	10	2
28	2		3	9	9	5	40	9	3	16	9	3
2	3		3	5	1		21	6	2	5	8	
31	2		3	13	9	4	50	12	2	18	16	2
6			1	3	2		9	2	1	5	1	
32	2		3	13	10	4	49	10	3	20	13	3
7			1	4	1	1	12	4		4	4	
23	2		4	9	4	4	38	7	1	15	13	2
16				7	8	1	21	7	2	8	3	1
4			1	3			6	1	1	3	1	

Hinsicht können auch zwischen den Gruppen der schweren (5 bzw. 6 positive Ergebnisse von 6 Funktionsprüfungen) und weniger schweren (3 bzw. 4 positive Resultate von 6 Bestimmungen) Hyperthyreose-Fällen keine Unterschiede beobachtet werden.

Die Ergebnisse der beiden Hyperthyreose-Gruppen — d. h. der beiden Hyperthyreose-Typen (einerseits dienzephalische Hyperthyreose, mit Exophthalmus verbundene Fälle und diffuse Strumafälle, anderseits Fälle ohne Exophthalmus, bzw. Knotenstrumen) — konnten infolge der geringen Zahl der in Gruppe II eingereihten Fälle nicht verglichen werden. In der Spalte EEG-V befinden sich verhältnismäßig wenig Fälle aus der letzten Gruppe; zwischen dem dienzephalischen und hypophysären Typ sind keine Abweichungen zu beobachten. Auf Grund des EEG-Befundes können also hinsichtlich des Typs der Hyperthyreose keine Anhaltspunkte gewonnen werden.

Die Untersuchung der neurophysischen Erscheinungen führt zu ähnlichen Ergebnissen: Unter den somatischen Angaben befinden sich die meisten Fälle in sämtlichen Gruppen in den Säulen 4 und 5, während unter den psychischen Faktoren die Mehrzahl der Fälle in die 1., 3. und 4. Säule gehört.

Besprechung

Bei der Bewertung der Fälle muß der zwischen Nervensystem und Schilddrüsenfunktion bestehende enge und mannigfaltige Zusammenhang in Betracht gezogen werden. Die Funktionen der beiden Systeme können einander unter normalen Verhältnissen, aber auch bei Regulationsstörungen beeinflußen (Steigerung, Verminderung) oder aber es kann sich zwischen ihnen eine Kompensation bzw. Gegenregulation entwickeln.

Die zwischen Nervensystem und Schilddrüsenfunktion bestehende enge Verbindung bzw. Wechselwirkung kann bekanntlich durch verschiedene Mechanismen zur Geltung kommen. Bei den Hyperthyreose-Typen z. B., bei denen die primäre Erscheinung annehmbar die Steigerung der Schilddrüsenfunktion ist (also nicht kortikalen oder dienzephalischen Ursprungs, wie z. B. Thyreoiditis), kann die Funktionsstörung des Nervensystems — die sekundär, infolge Thyroxin bzw. Schilddrüsenhormonwirkung entsteht — ebenfalls häufig nachgewiesen werden. Der Angriffspunkt des Thyroxins ist außer dem peripheren Gewebe bekanntlich das Nervensystem, in erster Reihe das Zentralnervensystem. Die Schilddrüse kann demzufolge durch gesteigerte Thyroxin- bzw. Hormonausscheidung die Reizbarkeit des vegetativen Nervensystems erhöhen [9, 14, 17, 27].

Es ist aber ebenfalls bekannt, daß bei gewissen Formen vegetativer Reizstörungen [»sympathische Hypertonie« (3)] nach gewisser Zeit eine gesteigerte Schilddrüsenfunktion zustandekommen kann [22].

Im sog. Feedback-Mechanismus der Schilddrüse — die ein unter zentraler Leitung stehendes, peripheres endokrines Organ ist — kommt außer dem hormonalen Einfluß wahrscheinlich auch die neurogene Vermittlung zur Geltung. Die veränderte Funktion des Zentrums kann aber wieder die Schilddrüsenfunktion beeinflussen.

Durch Zerstörung des Habenula-Gebietes konnte Mess [16] die infolge der Veränderung des Schilddrüsenhormonspiegels des Blutes in der TSH-Ausscheidung zustandekommenden Reaktionen hemmen.

In den verschiedenen klinischen Fällen sind also außer der gestörten Funktion der vegetativen Regulation und der Schilddrüse auch Störungen der hormonalen Regulation und des Nervensystems vorzufinden. Da zwischen diesen Funktionsstörungen ein enger und mannigfaltiger Zusammenhang besteht, können sie einander in bedeutendem Maße beeinflussen: Die vegetative

Regulationsstörung kann die Schilddrüsenhyperfunktion hervorrufen bzw. steigern, die Schilddrüsenhormone dagegen können durch Erhöhung der Reizbarkeit des Nervensystems zur Verschlimmerung der vegetativen Regulationsstörung beitragen und unter Umständen auch die auf die Schilddrüse ausgeübte schädigende Wirkung des Prozesses steigern. Auf diese Weise entsteht ein Circulus vitiosus bzw. ein klinisches Bild, in dem es kaum festzustellen ist, welche Funktionsstörung — die endokrine oder die des Nervensystems — die primäre war. In beiden Fällen kommt aber die erwähnte Wechselwirkung zur Geltung.

Ob die analysierten Prozesse bzw. die komplizierten, unter Beteiligung beider Systeme entstandenen klinischen Bilder primär durch die Schilddrüse hervorgerufen wurden, oder ob sie infolge der gestörten Nervensystemfunktion zustandekamen, konnten wir — in Anbetracht dieser mannigfaltigen Funktionsstörungen — mit den angewandten Untersuchungsverfahren (EEG, neuropsychiatrische Methoden) nicht eindeutig feststellen. Diese Verhältnisse — die mit unseren Ergebnissen übereinstimmen — unterstützen die Hypothese, laut der im Entstehen bzw. in der Pathogenese gewisser Hyperthyreose-Gruppen der vegetativen Regulationsstörung als Initialmechanismus eine wesentliche Rolle zukommen kann. Diese, zu Beginn hauptsächlich Nervensystemfunktionsstörung übt unter Einwirkung gewisser Verhältnisse auch auf die Schilddrüse einen pathologischen Einfluß aus, worauf, die Schilddrüse anfänglich kaum bemerkbar, später aber immer ausdrücklicher hyperfunktioniert: Durch die verschiedenen Stadien des Präbasedows kann sich auf diese Weise das typische Bild der Hyperthyreose entwickeln [23].

LITERATUR

1. Bansi, H. V., Kracht, J., Kracht, M., Meissner, J.: Dtsch. med. Wschr. **78**, 256 (1953).
2. Becka, D. R., Condon, J. V., Gibbs, F. A.: Electroenceph. clin. Neurophysiol. 1954.
3. Birkmayer, W., Winkler, W.: Therapie der vegetativen Function, Springer, Wien, 1951.
4. Charcot, J. M.: Gaz. méd. Paris (1856).
5. Chvostek, F.: Wien. klin. Wschr. (1910).
6. Dongier, M.: Psychosom. Med. **18**, 310 (1956).
7. Eickhoff, W.: Dtsch. med. Wschr. **76**, 171 (1951).
8. Haynal, I.: Magy. Tud. Akad. Biol. orv. Tud. Osztály. Közl. **6**, 307 (1955).
9. Issekutz, B., Hetényi, G. jun.: Arch. int. Pharmacodyn. **78**, 512 (1949).
10. Jendrassik, E.: Orv. Hetil. (1885)
11. Lindz, Th.: Psychosom. Med. **11**, 2 (1949).
12. Lindsley, D. B., Rubinstein, B. B.: Proc. Soc. exp. Biol. (N. Y.) **35**, 558 (1937).
13. Mandelbrote, B. M., Wittkower, E. D.: Psychosom. Med. **17**, 109 (1955).
14. Mansfeld, G.: Die Hormone der Schilddrüse und ihre Wirkungen. Schwabe, Basel, 1943.
15. Marie, P.: zit. 3.
16. Mess, B.: IV. International Goitre Conference, London 1960. Excerpta med. Foundation, New York.
17. Meyer, H. H.: Dtsch. med. Wschr. **57**, 1531 (1931).
18. Milcu, St. M., Brosteanu, E., Jonescu, B., Nicolescu-Catargi, A.: Endocrinologia (Buc.) **1**, 26 (1956).
19. Milcu, St. M., Cohen, E.: Stud. Cercet. Endocr. **1—2**, 275 (1955).

20. Milcu, St. M., Demetrescu, M., Belloiu, D.: ref. Electroenceph. clin. Neurophysio. **14**, 568 (1962).
21. Moussong-Kovács, E., Mike, T.: Orv. Hetil. **101**, 1133 (1960).
22. Policzer, M., Bazsó, E., Marton, M.: Orv. Hetil. **103**, 930 (1962).
23. Policzer, M., Fiala, E., Mike, T., Balassa, M.: Acta med. Acad. Sci. hung. **16**, 205 (1960).
24. Policzer, M., Fiala, E., Mike, T., Balassa, M., Sáfrány, L.: Orv. Hetil. **100**, 785 (1959).
25. Policzer, M., Földes, J., Sáfrány, L., Balassa, M.: Sugárzó izotópok hazai felhasználása, Budapest **1**, 65 (1958).
26. Rubin, M. A., Cohen, L. H., Hoagland, M.: Endocrinology **21**, 536 (1937).
27. Schittenhelm, A., Eisler, B.: Z. ges. exp. Med. **86**, 275 (1933).
28. Szabó, G., Kertai, P., R. Balogh, I., Kneiszl, F.: Kíséri. Orvostud. **7**, 127 (1955).
29. Szentágothai, J.: Orv. Hetil. **100**, 57 (1955).
30. Thiebaut, F., Rohmer, F., Wackenheim, A., Kurtz, D.: Presse méd. **66**, 1323 (1958).
31. Thiebaut, F., Rohmer, F., Wackenheim, A.: Electroenceph. clin. Neurophysiol. **10**, 1 (1958).
32. Uotila, N. N.: Endocrinology **26**, 129 (1940).
33. Vague, J., Gastaut, H., Codaccioni, J. L., Roger, A.: Ann. Endocr. (Paris) **18**, 996 (1957).
34. Wedler, H.: Stammhirn und innere Erkrankungen, Springer, Wien, 1953.
35. Wolfshaut, C., Ionescu, B.: Stud. Cercet. Endocr. **2**, 75 (1951).

Dr. Miklós Policzer, Budapest VIII. Vas u. 17. Ungarn
Dr. Erzsébet Moussong-Kovács, Budapest VIII. Balassa u. 6. Ungarn
Dr. Emma Bazsó, Dr. Mihály Marton } Budapest VIII. Vas u. 17. Ungarn

THE NUCLEIC ACID CONTENT OF THE LENS AND SOME PROPERTIES OF ITS "SOLUBLE RNA"

By

G. J. Köteles, F. Antoni and Magda Radnót

Institute for Radiobiological Research, Budapest, and First Department of Ophthalmology, University Medical School, Budapest

(Received February 18, 1963)

The acid-soluble ribose, lipid-phosphorus, protein and nucleic acid contents of the crystalline lens have been studied. The distribution of ribonucleic acid in the fresh lens has been found to differ from that in the frozen lens. The heterogeneity of the ribonucleic acid of the particle-free cytoplasm has been studied on ECTEOLA column. The properties of the fractions eluted at low NaCl concentration have been characterised on the basis of UV analysis, dialysis, gel-filtration and enzymatic digestion. The ribonucleic acid of the lens, prepared with phenol, has a component of low molecular weight, that can be eluted from the ECTEOLA column with 0.2 M NaCl, it shows an UV spectrum similar to that of nucleic acids, and contains ribose and phosphorus. The biological activity of this fraction is unclear.

Both morphologically and functionally, the crystalline lens occupies a special position among the organs of higher animals (vertebrates, mammals). In the course of ontogenesis it soon develops from the ectoderm, and with the development of its capsule it becomes isolated early. It is an avascular organ, has no innervation and its only contact with the organism through the capsule is humoral. According to the present view, the lens serves only to provide for an adequate refractory medium [1], ensured by the transformation of epithelial cells into special lenticular fibres and by the synthesis of special proteins. As compared to the mass of lens, that function is fulfilled by a small quantity of epithelial cells located subcapsularly at the anterior pole, in one layer and in the equatorial area. Under physiological conditions these cells divide at different rates [2], thus, they cannot be considered to be of the same type. The cells of the equatorial area gradually develop into lenticular fibres. Information relative to this process has been obtained chiefly by histological methods [33], the accompanying biochemical processes are unknown, and it is unresolved whether after the loss of the nucleus and persistence of the membrane these fibres have some kind of metabolic activity. Morphologically, and presumably also biochemically, these fibres differ from one another, because in the course of ontogenesis the fibres which developed earlier shift toward the inside of the lens. This is how the nucleus of the lens develops, which contains embryonic proteins not occurring anywhere else [3, 4].

The abbreviations used in this paper are: RNA = ribonucleic acid, RNA-P= ribonucleic acidphosphorus, DNA = desoxyribonucleic acid, DNA-P = desoxyribonucleic acid phosphorus, PCA = perchloric acid.

By means of fractionation procedures (chromatography, immune electrophoresis) the protein content of the lens could be separated into more and more components. For example, from the rabbit's lens five components were isolated by continuous flow electrophoresis by WOOD et al. [5], and from the lens of the human newborn FRANÇOIS and RABAEY [6] separated 13 components by agar electrophoresis. PAPACONSTANTINOU et al. [7] broke up the alpha-crystalline prepared from the nuclear and cortical fibres of the bovine lens into further four fractions by DEAE-cellulose chromatography and characterised them by immunechemical methods. Yet, it seems that, as compared with other organs, the lens contains few qualitatively different protein types. It is particularly remarkable that, although the antigenic components of the lens increase in number in the course of phylogenesis, only slight differences exist in the antigenic patterns of closely related species [8].

The role in protein synthesis of nucleic acids has been amply proved. In view of those outlined above, the lens seems to be a suitable object for studies on the synthesis of specific proteins and nucleic acids, and on the biochemical mechanism and control of cell division. It is namely known, on the one hand, that after exposure to noxious effects (mechanical trauma, UV, and ionising radiations) the lens must regenerate without external cellular help, since the capsule does not permit the entry of cells; on the other hand, the division of epithelial cells is a light-dependent procedure, the mitotic index being higher in the dark, than in light [2]. The latter observation suggests the existence in the lens of a light-reactive mechanism regulating nucleic acid synthesis, that may exert its effect not only on the lens, but humorally on the organism as a whole, and thus, together with nervous perception of light beginning in the retina, the lens, too, may take part through a humoral mechanism in the genesis and control of the diurnal and nocturnal biorhythm of the organism. The existence of a light-dependent regulating mechanism, unusual with mammalian cells, seems to be corroborated by the recent evidence published by CREMEL-BARTELS [9] that the pteridines isolated from the lens showed differences in spectrum and other properties before and after exposure to sunshine.

Information concerning the nucleic acid metabolism of the lens, and in particular the synthesis of nucleic acids and specific proteins, may shed some light on the mechanism of cataract development. This is why we have undertaken to study the nucleic acid metabolism of the lens.

In a previous paper [10] we published data as to the quantitative relations of some biochemical components of the lens, and gave an account of the heterogeneity of the RNA prepared from the lens by phenol method and of the changes in the chromatographic pattern upon ribonuclease digestion.

In the present paper we shall report, on the basis of a considerable number of data, on the relative concentrations of some biochemical components building

up the lens, with special reference to nucleic acids, as well as on some properties of the RNA found in the supernatant fraction (S-fraction) obtained by centrifugation of the lens homogenate at 105 000 g.

Methods

Experimental object

The lenses were obtained from inbred rabbits, 3 to 4 months old, immediately after decapitation.

Biochemical analysis

The lenses were placed into conical centrifuge tubes and homogenized in distilled water at + 4°C with a glass rod. From the homogenate the nucleic acids were isolated by the Schmidt—Thannhauser method modified for micro-work [11, 12]. The ribose contents of the acid soluble and RNA fractions were determined by the orcin colour reaction[13], using a 10 μg/ml ribose solution as the standard. The quantity of RNA-P was computed from the ribose contents of the RNA fraction, on the basis of the evidence that in the RNA prepared from the lens with phenol the ratio of orcin-reacting ribose and phosphorus was 2.4. Phosphorus was determined by the method of GRISWOLD *et al.* [14]. Protein was determined by the method of LOWRY *et al.* [15], using bovine serum albumin as standard. DNA was determined on the basis of the indole colour reaction [16], using calf thymus DNA prepared according to KAY, SIMMONDS and DOUNCE [17] as standard. DNA could not be determined by the indole colour reaction from the alkaline solution of the RNA-freed precipitate obtained with the Schmidt—Thannhauser method, partly because a very intense pink colour resulted in the course of developing the indole colour reaction, that was very difficult to remove even by the usual shaking-out with chloroform and therefore no reproducible results were obtained, partly because this way we obtained improbably low DNA values. For this reason we extracted previously the DNA with 1 n PCA at 100°C for 45 minutes from the fraction remaining after the removal of RNA-nucleotides and containing protein and DNA. In this case, too, the aspecific colour mentioned above made its appearance, but it could be eliminated by shaking out four times with chloroform. The results thus obtained were well reproducible. As to the disturbing aspecific colour, it should be pointed out that a more intense colour resulted when the S-fraction of the lens homogenate was analysed as described above than the one obtained in the precipitate; it is supposed that the indole reaction had been interfered with by protein, carbohydrate or some other substances contained mainly in the S-fraction.

The heterogeneity of RNA was studied in RNA prepared by KIRBY's phenol method [18] on ECTEOLA cellulose column (0.41 meq/g, SERVA) by stepwise elution, described in detail elsewhere [10].

Gel filtration was done by means of Sephadex G—25, Medium (Pharmacia, Uppsala, Sweden).

For ribonuclease digestion, calf pancreas ribonuclease (609 units/mg) [34] was used. The experimental conditions are described in the chapter on Results.

The lenticular homogenate was fractionated in a Spinco L preparative ultra centrifuge. A spectrophotometer of the Unicam SP 500 type was used for the photometric measurements.

Results

The results for the nucleic acid content as well as for the acid-soluble ribose, lipid-P and protein contents determined from the different fractions obtained by the Schmidt-Thannhauser method, are shown in Table I.

On the basis of the results in Table I, relating the single components to DNA-P, it can be seen that the value for the RNA-P/DNA-P ratio is around 6; the ratio for protein/DNA-P, $2 \cdot 10^4$; that for lipid-P/DNA-P, around 5;

Table I

Acid-soluble ribose, lipid-P, protein, RNA-P and DNA-P contents of the rabbit lens

	Weight g	Acid-soluble ribose μg	Lipid-P μg	Protein μg, 10^{-3}	RNA-P μg	DNA-P μg	RNA-P / DNA-P
Mean	0.3912	330	24.4	100	30.3	5.19	6.60
S. D.*	0.0348	66	2.97	4.6	2.98	0.99	1.39
Values min. and and max.	0.3314— 0.4385	171—416	16.2— 30.8	81—142	24.7— 36.7	3.09— 7.28	4.71— 8.90
Number of estimations	25	35	31	25	57	32	32

$$*_{S.D.} = \sqrt{\frac{\Sigma d^2}{n-1}}$$

and that for acid-soluble ribose/DNA-P, around 64. As compared with other mammalian organs [19], in the lens the RNA-P/DNA-P and the protein/DNA-P ratios are very high, while the lipid-P/DNA-P and the acid-soluble ribose/ /DNA-P ratios are low [20]. The unusual high value for the protein/DNA-P ratio is of course, since a small part of the lens is made up by cells, while the rest is composed of protein fibres having no nuclei. We have not attempted to analyse the lipid content and composition of the lens. The value for lipid-P relates to the phosphorus content of the lipid fraction. This supplies information merely as to the quantity of P-containing lipids, but part of it might not originate from lipids.

Because of the unusually high RNA/DNA ratio we studied the ratio of soluble RNA and RNA bound to subcellular particles, furthermore the special character of the lenticular RNA.

Therefore 10 lenses isolated immediately after decapitation, as well as 10 other lenses stored at —15° C for a few days, were homogenized in 0.25 M sucrose solution in a Potter-Elvehjem glass homogenizator at 2000 r. p. m. for 5 minutes, then centrifuged in Spinco I preparative ultracentrifuge for 90 minutes at 35 000 r. p. m. (40 rotor). The supernatants and the precipitates thus obtained were analysed by the method described. The results are presented in Table II.

Table II shows the values for the lipid-P, protein and RNA-P contents of the supernatants obtained after exposure of the homogenate to 105 000 g (S-fraction) and the precipitates isolated from fresh and frozen lenses, as well as (in parenthesis) the percentage distribution related to the total amounts of the single components of the initial homogenate. The experiments were repeated several times, and although the differences in initial total amount corresponded to the data in Table I, the values concerning percentage distribution proved to be reproducible within the limits of error.

Table II

Lipid-P, protein and RNA-P contents of the precipitate and supernatant fraction obtained after centrifugation the lens homogenate at 105 000 g

Lens	Lipid-P μg	Protein μg	RNA-P μg
Precipitate	224.0	290.0	156.6
Fresh	(89)	(25)	(59)
Supernatant	29.1 (11)	820.0 (75)	108.0 (41)
Precipitate	224.0	113.0	72.3
Refrigerated	(89)	(14)	(26)
Supernatant	30.0 (11)	707.0 (86)	206.8 (74)

In parentheses: percentage distribution related to total amount of single components

The data in Table II reveal that in the fresh lens the S-fraction contains 11 per cent of the lipid-P, 75 per cent of the protein and 41 per cent of the RNA. After storage in the frozen state, the percentage ratio of lipid-P is unchanged, that of protein increases to 86 per cent, and that of RNA to 74 per cent, in the supernatant. These data indicate that about half of the lenticular RNA is "soluble RNA", and that on freezing and thawing the lens an even greater percentage of the total RNA passes over into the S-fraction.

In a previous report [10] we have described the heterogeneity on the Ecteola column of the RNA isolated with phenol from the whole lens stored in the frozen state, and the chromatographic changes following digestion of the RNA with ribonuclease. The amounts of the fractions eluted with solutions of low NaCl concentration were considerable and after ribonuclease treatment the fractions which before were elutable only with alkaline eluents became possible to elute with NaCl. On the basis of the results presented in Table II we have tried to assess the chromatographic difference of the RNA found in the S-fraction of the fresh lens from that of the RNA prepared from the whole lens, furthermore to obtain more detailed information as to the properties of the single fractions.

The chromatogram of the RNA obtained after phenol treatment of the S-fraction of fresh lenses homogenised in 0.25 M sucrose is shown in Fig. 1.

Fig. 1 shows that about 60 per cent of the RNA prepared by phenol can be eluted from the column with solutions of different NaCl concentration, and a further 40 per cent with alkaline solutions of different concentrations. As compared with the chromatogram of the RNA prepared from frozen whole lenses [10], there is no qualitative and very little quantitative difference, inasmuch as here the relative concentrations of the fractions eluted with alka-

line eluents are lower. In RNA preparations obtained by the same method from other mammalian cells [21, 22] we have not found such remarkably great quantities of fractions elutable at low NaCl concentration. The question emerged also on the basis of the experiments with ribonuclease [10], as to whether these were degradation products of RNA produced upon the effect of endogenous ribonuclease, or components existing in the lens *in vivo*, too, that can be prepared together with RNA. To find the solution to this question, we have carried out investigations to characterise the isolated fractions.

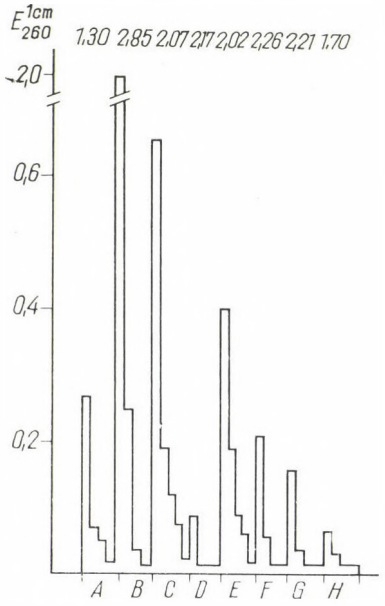


Fig. 1. The heterogeneity of RNA contained by the supernatant fraction of lens homogenate, after centrifugation at 105 000 g; on Ecteola column, by stepwise elution. The letters mean the different eluents, as follows: A: phosphate buffer, 0.01 M, pH 6.85; B, C, D: 0.2, 0.6, 1.0 M NaCl in phosphate buffer, respectively; E, F, G: 0.1, 0.2, 0.6 N NH_4OH in above phosphate solution containing 1.0 M NaCl, respectively; H: 1.0 N NaOH

The numbers above the columns show the values of E_{260}/E_{280} ratio. With each eluent, 5 ml fractions were eluted, to a total of 25 ml

The fraction elutable with 0.2 M NaCl was dialysed, rechromatographed and finally digested with ribonuclease. The fraction was dialysed overnight against a phosphate buffer at +4° C. The fraction proved to be non-dialysable. Even after dialysis against 2 M NaCl for 20 hours merely 25 per cent were dialysed out. In the course of rechromatography after dialysis the fraction mentioned was to be eluted again with 0.2 M NaCl solution. Treatment with ribonuclease did not alter the rechromatographic appearance of this isolated fraction. Treatment with ethanol containing potassium-acetate, commonly

used for the precipitation of RNA, caused only 15 per cent of the isolated fraction to precipitate. On ground of all these findings we have concluded that we were dealing with an oligonucleotide of relatively low molecular weight. Therefore we subjected the isolated fraction to gel filtration, in order to determine the approximate molecular weight. The material applied to Sephadex G-25 was found to possess just one component, one of low molecular weight corresponding to the specification of the gel filter used, notably one lower than 4500 in molecular weight. We wanted to confirm this fact also by gel-filtrating the total RNA preparation on Sephadex G-25.

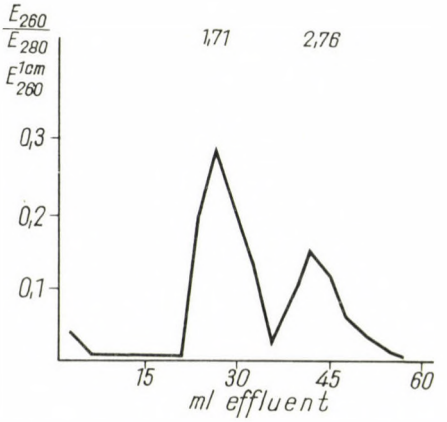


Fig. 2. Gel filtration of RNA prepared from the supernatant of lens homogenate after centrifugation at 105 000 g, on Sephadex G-25 column

Fig. 2 reveals that on the basis of O. D. at 260 mμ about 65 per cent of the material applied to the Sephadex G-25 column proved to be higher than approximately 4500 and 35 per cent was lower than 4500 M. W. The percentual amount of the latter component in the RNA preparation was the same as that of the fraction eluted from the Ecteola with 0.2 M NaCl solution. The E_{260}/E_{280} ratio of this low molecular weight fraction is 2.8, 11.8 μg of ribose corresponding to 1 E_{260}, as opposed to the other nucleic acid fractions which show values of 2.0 and about 9.9, respectively.

In the next step we rechromatographed the fraction eluted from the Ecteola column with 0.6 M NaCl. Of that, 69 per cent was eluted again with 0.6 M NaCl, while 35 per cent with 0.2 M NaCl. Closely similar results were obtained after gel-filtrating the isolated 0.6 M NaCl fraction on Sephadex G-25 (Fig. 3).

Fig. 3 reveals that after gel-filtration of the fraction eluted from the Ecteola with 0.6 M NaCl, we obtain a fraction higher than 4500 and one lower than 4500 in molecular weight. In the case of both fractions the E_{260}/E_{280}

ratio showed values characteristic of nucleic acids. The rechromatographed 0.6 M NaCl fraction was digested with ribonuclease (for 15 minutes at 37° C, in 0.01 M, pH 6.85 phosphate buffer medium, with 0.78 μg/ml enzyme); after rechromatography, 55 per cent of it could be eluted with the 0.6 M NaCl eluent. As shown in the previous paper [10], the fraction eluted by 0.6 M NaCl is detectable even after treating the lenticular RNA with ribonuclease for 3 hours. In addition, if digestion was continued for 24 hours, or if instead of RNA the lenticular homogenate was digested and RNA prepared after this was chromatographed, beside the fraction eluted with the 0.2 M NaCl eluent also that eluted by 0.6 M NaCl was detectable. Thus, this fraction proved to be relatively resistant to ribonuclease. This has also been proved by the following experiment.

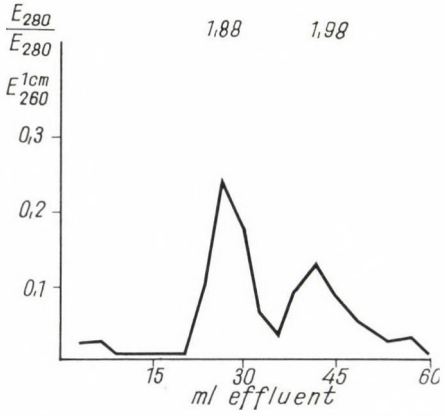


Fig. 3. Gel filtration on Sephadex G-25 column of the lenticular RNA fraction eluted from Ecteola column with 0.6 M NaCl solution

The RNA preparation isolated from the S-fraction of the homogenate was applied to an Ecteola column, then eluted directly with 1 M NaCl. Thereby the fractions elutable with NaCl were separated from those elutable with alkaline eluents; the latter remained on the column. Then the column was washed with phosphate buffer to free it from salts, 20 μg/ml of ribonuclease dissolved in buffer was allowed to flow through it for 90 minutes at a rate of 1 ml/min, then it was washed with distilled water. Subsequent elution was begun again with buffer, by the stepwise technique. The chromatogram thus obtained is shown in Fig. 4.

Fig. 4 reveals that the nucleic acid fractions which had remained on the column after elution with 1 M NaCl (broken line) turned into fractions eluted with 0.2 and 0.6 M NaCl (continuous line) in response to treatment with ribonuclease. It is also visible that the E_{260}/E_{280} ratio for the fractions thus obtained corresponded to that for the initial nucleic acids. This suggests that

the fraction originally present in the RNA preparation and eluted with 0.2 M NaCl is not the same fraction as that eluted with 0.2 M, arising after the breakdown of nucleic acids.

Discussion

As compared to its mass, the lens contains few cells. By the Schmidt—Thannhauser method adapted to micro-work the RNA and DNA contents of a single lens could be determined.

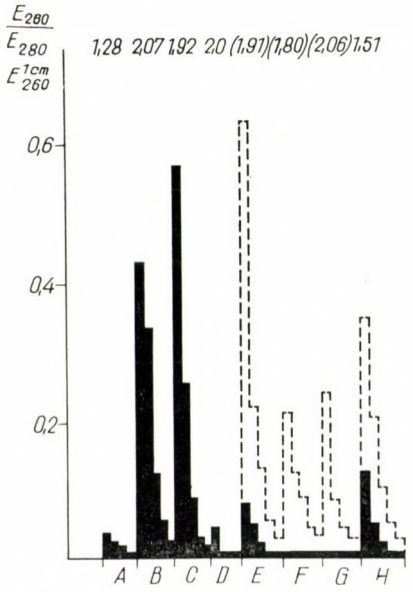


Fig. 4. Conversion of the lenticular RNA fractions elutable by alkaline eluents (broken line) to fractions eluted by eluents of low NaCl concentration (solid line), in response to ribonuclease treatment, on Ecteola column. The letters mean the same as in *Fig.* 1. Experimental conditions see in text

The DNA-P content of the lens has been found to be 5.2 μg. DAISLEY [23] determined by a similar method the DNA content of chick embryo lenses. It is known that within the same species the different types of somatic cells contain the same amounts of DNA. In the different types of inflammatory cells of the rabbit the average DNA-P content was found to be 0.6 μg [21, 24]. From this it may be concluded that the DNA-P content of the lens is equivalent to that of about $6 \cdot 10^6$ to $8 \cdot 10^6$ nuclear cells.

We found that one lens contained 30.3 μg of RNA-P. This amount agrees well with those reported by other authors [25, 26, 27]. The value reported by

VAN HEYNINGEN et al. [28] is lower, but these authors extracted the RNA by phenol-method. It is known, however, that although phenol makes most of the RNA water-soluble, there still remains a portion not released by the usual phenolic treatment, i. e. it remains in the phenolic phase. The amount of the latter varies from tissue to tissue. The lens, too, contains an RNA fraction not releasable by phenol, and thus the amount of RNA determined by this way cannot be considered to be the total RNA contained in the lens.

As compared with other mammalian organs, the lens showed extremely high RNA-P/DNA-P values and in the S-fraction high RNA content. This may be due to several factors. It is possible, on the one hand, that unlike the other organs, the lens contains really much more RNA than DNA, and, on the other, the lens may characteristically contain substances which are extractable together with RNA and imitate the behaviour, colour reactions and optical properties of RNA. The first possibility is supported by the evidence reported by MANDEL et al. [29], according to which RNA is demonstrable in the lens even in such areas lacking cellular structures. This may be explained by the fact that the fibrous matter of the lens arises from the epithelial cells at a certain stage of their development. In the course of this they lose their nuclei, i.e. DNA content, but a proportion of RNA may remain in the fibres, or even the synthesis of RNA may continue after the cellular structure has disappeared. This may be one of the explanations also for the high "soluble" RNA content of the lens. We have shown that in fresh lenses the total RNA/soluble RNA ratio is about 2.4, whereas in the case of lenses stored in the frozen state this value is around 1.3. Thus, in the course of storing in the frozen state the amount of "soluble" RNA is significantly increased. This may be explained partly by a damage to subcellular particles suffered during freezing, and partly by an effect of endogenous ribonuclease prior to freezing and on thawing, or by a combination of the two. But, PIRIE [30] found the ribonuclease activity of the lens to be lower than that of the liver. DISCHE et al. [31] found no difference in the relative concentrations of the RNA fractions of rat lenses during freezing and storing. However, the authors quoted before fractionation homogenized the lenses either with distilled water or with hypotonic solution. Under such conditions the values obtained for the total RNA/soluble RNA ratio should be viewed with criticism. We think that the total RNA/soluble RNA ratio can be influenced artificially, so for instance by freezing and by the choice of the homogenization medium. We have found a lower ratio when isotonic NaCl solution was used for homogenization than in the case of using sucrose for this purpose (unpublished data). Although we could demonstrate no significant differences in the total RNA content of the lens and in the heterogeneity of the RNA obtained from the S-fraction on the Ecteola column after storage in the frozen state, we still think it advisable to use fresh lenses to study the intracellular distribution and metabolism of RNA.

We have mentioned the presence of substances imitating the RNA characters, as the second possibility of explaining the conspicuously high RNA/ /DNA ratio of the lens. Concerning this we have shown that the RNA preparations obtained from the S-fractions of fresh and frozen lenses alike contained remarkably great amount of such "RNA"s, which could be eluted with solutions of low NaCl concentration. We had very strong doubts as to the nucleic acid nature of these fractions. We have therefore subjected these fractions to rechromatography, gel filtration and ribonuclease digestion.

The fraction eluted with 0.2 M NaCl could be rechromatographed on Ecteola column. Its approximate molecular weight on Sephadex G-25 proved to be lower than 4500, and it was not digested by ribonuclease.

Of the fraction eluted with 0.6 M NaCl, 65 per cent could be rechromatographed with 0.6 M NaCl, and 35 per cent was eluted with 0.2 M NaCl. A similar percentage ratio was obtained on gel filtration. After digestion with ribonuclease, 55 per cent of the 0.6 M NaCl fraction could be eluted again from the column with 0.6 M NaCl. Thus, while the 0.2 M NaCl fraction was absolutely resistant to ribonuclease digestion, 55 per cent of the 0.6 M NaCl fraction proved to be relatively resistant. The latter fraction could be demonstrated after 24-hour digestion of the RNA and of the lens homogenate. Both fractions were positive for ribose by the orcin test, the E_{260}/E_{280} ratio had a value of 2.8 of the 0.2 M NaCl fraction, and that around 2.0 of the 0.6 M NaCl fraction.

On the basis of our results we do not consider the fraction eluted with 0.2 M NaCl, which is coprecipitated with RNA by ethanol, to be nucleic acid. Therefore we view the data found by us and other authors for the RNA content of the lens and for the RNA/DNA ratio with criticism.

The fraction eluted with 0.2 M NaCl should be subjected to more detailed analysis and its function, too, awaits elucidation. Recently, CREMER-BARTELS [9] has isolated from the lens pteridines having UV absorption; their E_{260}/E_{280} ratio was higher than 2.0, they contained ribose and phosphorus, and incorporated ^{32}P. They showed strong fluorescence and light-sensitivity, i.e. on exposure to light a change resulted in the spectrum around 400 mμ and by paper chromatography the fluorescent spot and the ^{32}P activity were separated. On paper chromatography (unpublished data) we, too, found the 0.2 M NaCl fraction to be fluorescent and even after acidic hydrolysis we could not detect in it the various bases in such relative concentrations as in the RNA-hydrolysate.

These data make it most likely that the fraction we prepared together with RNA and eluted from the column with 0.2 M NaCl is identical with the substance isolated by CREMER-BARTELS, or at least with one of its components. VAN HEYNINGEN [32] found alpha-crystalline to be contaminated with a nucleotide-like substance, which could be separated from the protein by gel-filtration on Sephadex G—25. It seems therefore that the lens contains

some nucleotide-like compounds, co-precipitating readily with proteins and ribonucleic acids alike. Its function *in vivo* is still unclear. However, the possibility that it is a light-sensitive pteridine, and, furthermore, that the pteridine derivatives, folic acid, for instance, are known to take part in the synthesis of purine and pyrimidine bases, as well as the evidence put forward by VON SALLMANN et al. [2], that the intensity of lenticular DNA synthesis is light-dependent, induce one to suspect that this compound has some connexion with nucleic acid synthesis and with the diurnal changes of the mitotic index in the lens.

Investigations are in progress relative to the biological activity of the 0.2 M fraction, as well as to the role played by the 0.6 M fraction in the synthesis of the proteins and oligopeptides of the lens.

Acknowledgements

We are indebted to *Dr. V. Várterész* for support and advice, to *Dr. L. Boross* for advice in connexion with gel filtration, and to *Mrs. E. Gera* for outstanding technical work.

REFERENCES

1. VAN HEYNINGEN, R.: in Davson, H.: The Eye. Vol. 1., Academic Press, New York, p. 213. 1962.
2. VON SALLMANN, L., GRIMES, P., MCELVAIN, N.: Exp. Eye Res. **1**, 449 (1962).
3. FUCHS, R., KLEIFELD, O.: Albrecht v. Graefes Arch. Ophthal. **158**, 29 (1956).
4. FRANÇOIS, J., RABAEY, M.: Arch. Ophthal. **57**, 672 (1957).
5. WOOD, D. C., MASSI, L., SOLOMON, E. L.: J. biol. Chem. **234**, 329 (1959).
6. FRANÇOIS, J., RABAEY, M.: Arch. Ophthal. **61**, 351 (1959).
7. PAPACONSTANTINOU, J., RESNIK, R. A., SAITO, E.: Biochim. biophys. Acta (Amst.) **60**, 205 (1962).
8. HALBERT, S. P., MANSKI, W., AUERBACH, T.: in The Structure of the Eye. Academic Press, New York. p. 249, 1961.
9. CREMER-BARTELS, G.: Exp. Eye Res. **1**, 443 (1962).
10. ANTONI, F., KÖTELES, G. J., RADNÓT, M.: Exp. Eye Res. **2**, 65 (1963).
11. SCHMIDT, G., THANNHAUSER, S. J.: J. biol. Chem. **161**, 83 (1945).
12. DAVIDSON, J. N., FRAZER, S. C., HUTCHISON, W. C.: Biochem. J. **49**, 311 (1951).
13. MEJBAUM, W.: Hoppe-Seylers' Z. physiol. Chem. **258**, 117 (1939).
14. GRISWOLD, B. L., HUMOLLER, F. L., MCINTYRE, A. R.: Analyt. Chem. **23**, 192 (1951).
15. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., RANDALL, R. J.: J. biol. Chem. **193**, 265 (1951).
16. CERIOTTI, G.: J. biol. Chem. **198**, 297 (1952).
17. KAY, E. R. H., SIMMONDS, N. S., DOUNCE, A. L.: J. Amer. chem. Soc. **74**, 1724 (1952).
18. KIRBY, K. S.: Biochem. J. **64**, 405 (1956).
19. DAVIDSON, J. N.: The Biochemistry of the Nucleic Acids. Methuen, London, 1960, P. 104.
20. RAMAIAH, T. R., ANTONI, F., HUTCHISON, W. C.: In preparation.
21. KÖTELES, G. J., ANTONI, F., SZABÓ, L. D.: Acta physiol. Acad. Sci. hung. **22**, 1 (1962).
22. VÁRTERÉSZ, V., KÖTELES, G. J., ANTONI, F., SZABÓ, L. D.: Int. J. Radiat. Biol. In press.
23. DAISLEY, K. W.: Radiat. Res. **11**, 271 (1959).
24. KÖTELES, G. J., ANTONI, F., HIDVÉGI, E. J.: Nature (Lond.) **195**, 704 (1962).
25. MANDEL, P., QUARANTA, C. A.: C. R. soc. biol. (Paris) **151**, 604 (1957).
26. DEVI, A., FRIEL, R., LERMAN, S.: Arch. Ophthal. **65**, 115 (1961).
27. VAN HEYNINGEN, R.: in Davson, H.: The Eye, Academic Press, NewYork, 1962, Vol. 1., P. 221.

28. VAN HEYNINGEN, R., WALEY , S. G.: Exp. Eye Res. **1**, 155 (1961).
29. MANDEL, P. et al. cit. DISCHE (31).
30. PIRIE, A.: Radiat. Res. **15**, 211 (1961).
31. DISCHE, Z., DEVI, A., ZELMENIS, G.: Amer. J. Ophthal. **51**, 993 (1961).
32. VAN HEYNINGEN, R., WALEY, S. G.: Exp. Eye Res. **1**, 336 (1962).
33. COGAN, C. D.: Exp. Eye Res. **1**, 291 (1962).
34. KALNITSKY, G., HUMMEL, J. P., RESNICK, H., CARTER, J. R., BARNETT, L. B., DIERKS, C.: Ann. N. Y. Acad. Sci. **81**, 542 (1959).

György J. KÖTELES, Ferenc ANTONI, Budapest XXII. Pentz K. u. 5, Hungary.
Prof. Dr. Magda RADNÓT, Budapest VIII. Illés u. 15, Hungary.

Printed in Hungary

A kiadásért felel az Akadémiai Kiadó igazgatója — Műszaki szerkesztő: Farkas Sándor

A kézirat nyomdába érkezett: 1963. VIII. 28. — Terjedelem: 8,25 (A/5) ív, 28 ábra

63.57652 — Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György

ACTA MEDICA

ТОМ XIX — ВЫП. 3

РЕЗЮМЕ

ОПЫТ ИССЛЕДОВАНИЯ ЛИМФАДЕНОГРАММЫ БОЛЬНЫХ СО ЗЛОКАЧЕСТВЕННЫМИ ОПУХОЛЯМИ

М. СЕГВАРИ и А. ИХРАЧКА

Статья содержит наблюдения авторов в связи с исследованием лимфаденограммы двух больных со злокачественными опухолями. Авторы обращают внимание на значение лимфаденографии в диагностике и при выборе соответствующего метода лечения.

ИССЛЕДОВАНИЕ МЕХАНИЗМА УЛЬЦЕРОГЕННОГО ДЕЙСТВИЯ БУТАЗОЛИДИНА

Л. ЧАЛАИ и Э. ТОТ

Авторы экспериментально выявили, что при даче различных доз бутазолидина можно дифференцировать действие этого препарата на надпочечники и на желудок. При ежедневной даче 100 мг/кг бутазолидина повреждения желудка наступают раньше, чем повышение кортикостероносинтетизирующей активности надпочечников Активацию надпочечников удалось выявить только дозами в 200 мг/кг бутазолидина. Эти наблюдения говорят за то, что в развитии действия бутазолидина на желудок решающую роль, играет не изменение функции надпочечников а другой фактор.

После этого авторы исследовали эффект бутазолидина на повышение секреции солянокислого гистамина. Они доказали, что после дачи 150 мг/кг бутазолидина в течение 10 дней секреция солянокислого гистамина повышается. Подобный эффект можно выявить только в начале дачи (на 5. день) большей дозы (220 мг/кг) бутазолидина. На 10. день животные, получившие бутазолидин, выделяют одинаковое количество соляной кислоты, как и контрольные животные. Прекращение повышения секреции соляной кислоты к концу дачи бутазолидина объясняется отчасти токсическим действием этого препарата а с другой стороны оно связано с отеком слизистой оболочки.

Хлорорит, снижающий секрецию соляной кислоты, не уменьшает ульцерогенное действие бутазолидина.

НОВЫЙ МЕТОД ДЛЯ ВЫЯВЛЕНИЯ ПРОИЗВОДСТВА ЭНТЕРОТОКСИНОВ СТАФИЛОКОККАМИ

И. НИКОДЕМУС, Л. КАНИЖАИ и Э. ШЕЛЛЕИ

В связи с семейным пищевым отравлением, вызванным Staphylococcus aureus, авторы применяли новый метод для выявления производства энтеротоксина микробами, выращенными из пищевых проб. Они кормили взрослых собак культурой исследуемого

штамма. Подопытные после 4—5 часов латентного периода реагировали на дачу болезнетворного агента частым выделением жидкого кала и менее выраженными общими симптомами. В отличие от симптомов заболевания у людей, рвота появлялась у собак только изредка. Симптомы продолжались 7—9 часов, причем болезнетворный агент в кале животных можно было выявить в течение 8—12 часов в большом количестве, а в течение дальнейших 12 часов — в меньшем количестве. Рвотные массы также содержали Staphylococcus aureus. Стафилококками, не являющимися болезнетворными, при подобных экспериментальных условиях не удавалось вызвать заболевания животных.

Авторы придерживаются того мнения, что описанный ими метод пригоден для выявления стафилококкового энтеротоксина, хотя он не равноценен пробе Дольмана.

КАРИОЗО-ИНТЕНСИВНОСТЬ У ВЗРОСЛОГО НАСЕЛЕНИЯ ВЕНГРИИ И ЕЕ СРАВНЕНИЕ С АНАЛОГИЧНЫМИ ДАННЫМИ ЧЕХОСЛОВАКИИ, США и ИТАЛИИ

П. БРУСТ

Автор приводит данные о кариозо-интенсивности почти 13 000 человек в возрасте выше 14 лет, без выбора, по пятилетним возрастным группам, а также по полам. В отношении всех обследований частота кариоза равна 96,8%, кариозо-интенсивность — 11,4, DMF-индекс, при среднем возрасте 40,8. Регистрация зубов мудрости повышает DMF-индекс в общем на 2,03.

Устанавливается, что кривая кариоза — почти прямая линия, и что кариоз у женщин во всех возрастных группах выше, чем у мужчин. Среди компонентов DMF-индекса перевес имеет кривая отсутствия зубов.

Сравниваются данные кариоза, собранные в других областях страны, с данными из окрестности города Бая, и устанавливается, что имеющиеся до сих пор данные — за исключением более высоких показателей, полученных в г. Сегед — в сущности аналогичны с данными г. Бая.

Ввиду того, что согласно результатам исслевдований, связь между возрастом и DMF-индексом проявляется в виде прямой линии, арифметрическое среднее DMF-индексов различных возрастных групп представляет собой величину, характерную для среднего возраста. Поэтому результаты исследований, охватывающих несколько возрастных групп, также можно начертить возле кривой кариозо-индексов, собранных автором.

После этого сравнивается кривая кариоза взрослого населения других 3 государств с венгерскими данными. Устанавливается, что кривые кариоза в США и Чехословакии проходят весьма близко друг к другу, выше венгерской кривой, в то время как итальянская кривая проходит ниже. Бросается в глаза приблизительно параллельный ход кривых. Из этого следует, что интенсивность поражения кариозом определенной группы населения уже в 15-летнем возрасте представляет собой характерную для страны величину. Если искать причину отклонений между интенсивностью кариоза у населения рассмотренных четырех стран, то она заключается лишь отчасти в различии питания: а именно, в потреблении сахара. Прочие факторы питания предоставляют противоречивые данные о величине кривых кариоза. Антикариогенное действие так наз. предохранительной пищи не видно из сопоставления. Общепринятую параллельность между степенью индустриализации, потреблением сахара и интенсивностью кариза можно установить и из данной статьи.

ДЕЙСТВИЕ МАГНИЯ НА АЛИМЕНТАРНЫЕ ИНФАРКТОИДНЫЕ ИЗМЕНЕНИЯ СЕРДЦА

Я. РИГО, Д. ШИМОН, Ч. ХЕДЬВАРИ и Й. ШОШ

При кардиопатогенной диете, дополненной магнием, авторы не наблюдали инфарктоидных изменений сердечной мышцы и снижения содержания калия и магния в сердечной мышце. Магний предотвращал и изменения содержания электролитов, наступающие при кардиопатогенной диете в сыворотке крови.

Содержание калия в перфузионной жидкости подвешенного по Штраубу выживающего сердца лягушки по истечении 15 минут достоверно меньше, если в питательной жидкости повышать содержание магния в 5 раз по сравнению с нормой.

На основании опытов, проведенных при помощи изотопа K^{42}, у животных, получавших богатую магнием диету, содержание K^{42} в сердечной мышце через 3 часа после внутривенного введения изотопа было на 20% выше, чем у контрольных животных.

ИССЛЕДОВАНИЕ СТЕРОИДОВ НА СТОЛБЕ С ФЛОРИСИЛОВЫМ АДСОРБЕНТОМ

I. Поведение различных стероидов на столбе «Серва»-флорисила

И. ФАРЕДИН

Автор проводил исследование стероидов с флорисилами «Серва», «Флука» и «Нимко» обладающими адсорбционным свойством, отличающимся от общеизвестных в литературе флорисилов. В опытах с кортизолом и кортизоном было установлено, что в условиях одинаковой очистки и одинаковой активации, между активностью флорисилов «Серва», «Флука» и «Нимко» наблюдается значительное различие.

Согласно результатам дальнейших исследований по обратному получению стероидов, прегнандиол, аллопрегнандиол, андростерон, этиохоланолон, изоандростерон и дегидроизоандростерон при исследовании в хлороформном растворе в «Серва»-флорисиле не абсорбируются, легко вымываются из него и их можно количественно получить обратно.

Результаты, по-видимому, подтверждают, что «Серва»-флорисил пригоден для отделения кортизола от кортизона, а также от 17-кетостероидов из биологических веществ.

При помощи метода, описываемого автором для регенерирования и активирования флорисилов, израсходованные флорисилы можно повторно регенерировать и хранить в течение нескольких недель при сохранении их активности.

ИССЛЕДОВАНИЕ СТЕРОИДОВ НА СТОЛБЕ ФЛОРИСИЛОВОГО АДСОРБЕНТА

II. Упрощенный метод для определения общего содержания 17-, 21-дигидрокси-20 кетостероидов в человеческой моче

И. ФАРЕДИН

Автор описывает пригодный для клинических серийных исследований, быстрый и точный метод для определения общего содержания 17-гидроксикортикостероидов в человеческой моче. Чтобы способствовать распространению метода, вместо спектрофотометрического измерения автор попытался применить определение при помощи простого фотометра Хавеманна. Описанный метод, по его мнению, пригоден для проверки функции коры надпочечников в клинической практике. Величины в норме: у женщин в возрасте 18—50 лет: 1,5—6,0 мг/24 ч. — у мужчин в возрасте 18—60 лет: 2,0—8,0 мг/24 ч.

ЭЛЕКТРОЭНЦЕФАЛОГРАФИЧЕСКОЕ И ПСИХОНЕВРОЛОГИЧЕСКОЕ ОБСЛЕДОВАНИЕ БОЛЬНЫХ ГИПЕРТИРЕОЗОМ

М. ПОЛИЦЕР, Е. МУССОНГ-КОВАЧ, Э. БАЖО и М. МАРТОН

Авторы на своем материале, состоящем из 46 случаев гипертиреоза и 45 случаев вегетативной дистонии, изучали ценность и используемость ЭЭГ и психоневрологических методов для характеристики неврологических отношений, патогенеза и клинических форм гипертиреоза.

Подчеркивается, что вследствие тесной связи между щитовидной железой и функцией нервной системы, при оценке результатов следует поступать с большой осторожностью. Выявленные при гипертиреозе отклонения сравниваются с группой больных,

страдающих расстройством вегетативной регуляции, а не со случаями здоровой, нормальной нервной регуляции (как это принято в литературе). Таким образом, эти изменения можно оценивать обособленно от изменений, не связанных с гипертиреозом, а вызванных функциональными расстройствами нервной системы различноы этиологии, возникающими в результате различных механизмов. При исследовании материала авторы установили, что

1. При гипертиреозе число аномалий ЭЭГ больше, чем при вегетативной дистонии, а определенные явления ЭЭГ даже в пределах этих аномалий имеют значительный перев с.

2. Перевес психических нарушений эмоционального характера также значителен в гипертиреотической группе.

3. Поведение данных ЭЭГ или психосоматических явлений нельзя связать ни тяжестью гипертиреоза, ни с решающей ролью результатов одного из функциональных проб щитовидной железы. С этой точки зрения нельзя придавать особое значение и величина основного обмена, превышающей +25%. Роль типа и клинической формы гипертиреоза в отношении результатов исследований — ввиду состава материала — не удалось выявить.

Критическое применение ЭЭГ и психоневрологических методов в целях дифференциальной диагностики «гипертиреозного синдрома» может предоставить нам данные для правильной оценки клинической картины.

ИЗУЧЕНИЕ СОДЕРЖАНИЯ НУКЛЕИНОВОЙ КИСЛОТЫ В ХРУСТАЛИКЕ И НЕКОТОРЫХ ОСОБЕННОСТЕЙ «РАСТВОРИМОЙ РИБОНУКЛЕИНОВОЙ КИСЛОТЫ»

Г. Й. КЁТЕЛЕШ, Ф. АНТОНИ и М. РАДНОТ

Изучалось содержание растворимых в кислоте рибозы, липоидного фосфора, белков и нуклеиновой кислоты в хрусталике. В распределении рибонуклеиновой кислоты наблюдается разница между свежими и замороженными хрусталиками. Гетерогенность содержания рибонуклеиновой кислоты в очищенной от частиц цитоплазмы изучалась на столбе ESTEOLA. Свойства фракций, извлекаемых при низкой концентрации хлористого натрия, определялись при помощи анализа ультрафиолетового спектра, диализа, фильтрации геля и энзиматического переваривания. Рибонуклеиновая кислота хрусталика препарированная фенолом, можно извлекать из столба ESTEOLA хлористым натрием в концентрации 0,2 М. Ее ультрафиолетовый спектр подобен спектру нуклеиновых кислот и она содержит рибозу и фосфор. Роль биологической активности этой фракции еще не выяснена.

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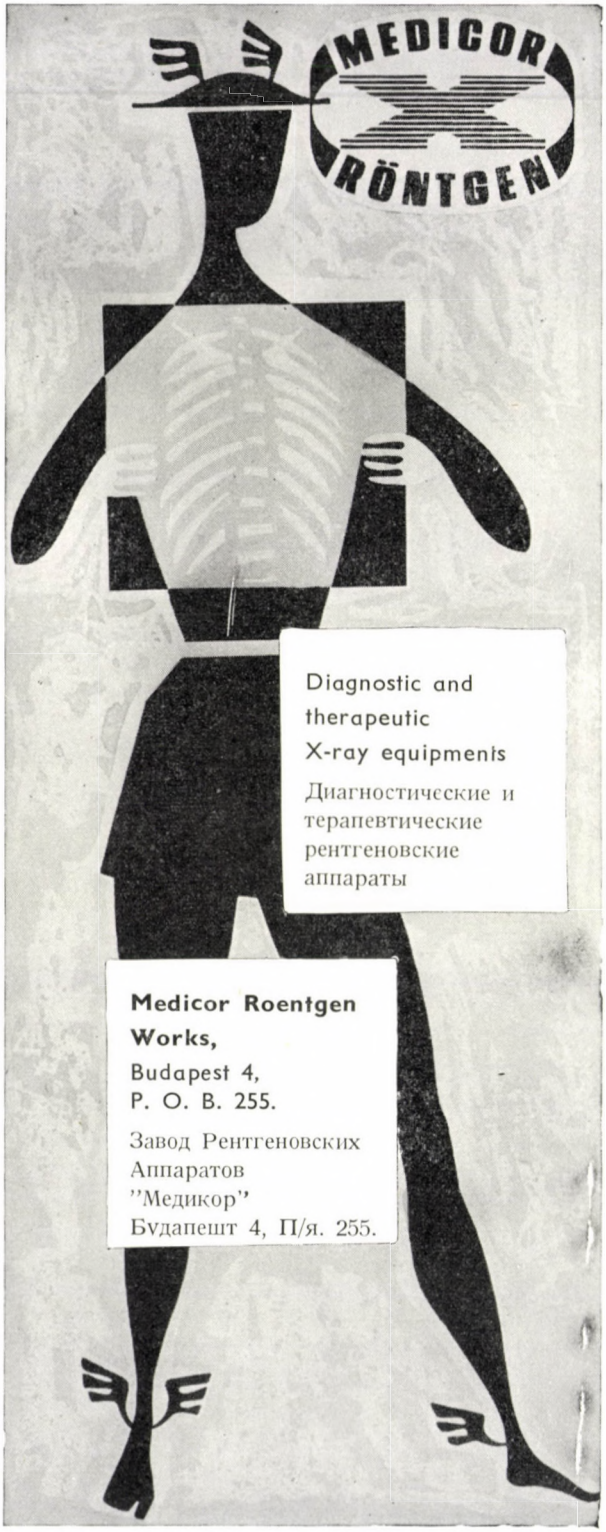
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Professor ISTVÁN RUSZNYÁK

President of the Hungarian Academy of Sciences, the Great Old Man of Hungarian Medicine, upon the event of his 75th year, extending their best wishes for happiness and further success in his life-long endeavours at the advancement and propagation of science.

EFFECT OF NORADRENALINE ON CEREBRAL CIRCULATION AND METABOLISM IN ISCHAEMIC SHOCK

By

GY. SZABÓ

(with the technical assistance of Mrs. L. Bertók and Miss Á. Prause)

INSTITUTE OF TRAUMATOLOGY, BUDAPEST (DIRECTOR: GY. SZÁNTÓ)

(Received May 10, 1962)

In the dog with experimental ischaemic shock, maintenance of blood pressure by the infusion of noradrenaline exerts no effect on survival. The rising blood pressure is accompanied by an increase of cerebral blood flow and oxygen consumption, but since cerebrovascular resistance is increased by noradrenaline, there is no linear relationship between the rises in blood pressure and blood flow.

In traumatic shock, its unique hypertensive properties appear to render l-noradrenaline eminently suitable for elevating a dangerously low blood pressure and thereby maintaining the circulation in the vital organs. The hypertensive effect of noradrenaline is due to an increase in peripheral resistance while cardiac output remains practically unchanged. In other words, the rise in blood pressure is of no advantage unless the increase in resistance, the vascular spasm, remains peripheral, and by not extending to the vital organs, positively improves the blood supply to them, prevents the development of irreversible lesions, and thus assists the organism in relieving and surviving the shock.

Earlier studies have shown that in experimental haemorrhagic [1, 6] and ischaemic [33] shock noradrenaline dilates rather than constricts the coronary arteries so that a rising blood pressure considerably increases the coronary circulation and the coronary fraction of cardiac output. Even so, noradrenaline has been found not to prolong survival [6, 8]; only FOZZARD and GILMORE [5] claimed to have made observations to the contrary. Accordingly, though it affects cardiac circulation favourably, the drug is unable to prevent death. This being the situation, it seemed promising to study the effect of noradrenaline on cerebral circulation and metabolism.

FRANK et al. [6] found that in experimental haemorrhagic shock noradrenaline promoted cerebral blood flow (observed through the sagittal sinus), but was without influence on cerebrovascular resistance. However, from blood flow alone it is not possible to conclude to the drug's effect on the metabolic disturbances associated with shock. In addition, it seemed necessary to test noradrenaline in some forms of shock other than haemorrhagic.

Material and methods

In dogs of both sexes, weighing 15 to 20 kg, under chloralose anaesthesia (0.10 g per kg) ischaemic shock was induced by circular compression of both hind legs in the region of the groin, by means of a tourniquet applied for 4 hours.

In view of the difficulties commonly encountered in measuring absolute cerebral blood flow in animal experiments, we contented ourselves with obtaining comparative values by observing blood flow in the carotid artery. To measure cerebral blood flow, KOVÁCH et al. [17] ligated the vertebral artery and connected a rotameter into both carotid arteries. As this procedure is not free from the limitation common to all methods involving the measurement of carotid flow — namely, that what they measure is not the cerebral but the cephalic blood flow, including muscles, skin, etc. — we refrained from ligating the vertebral artery and so avoided upsetting the equilibrium of cerebral circulation and exposing our animals to the trauma of previous surgery. The effect of noradrenaline on muscle, skin, etc. blood flow was studied with a bubble flowmeter connected to one of the brachial arteries. The femoral artery was unsuitable for this purpose since the hind legs of the animals had been rendered ischaemic.

Cephalic circulation was measured by means of a flowmeter connected to the carotid. Arterial pressure in the carotid was measured by means of a mercury manometer. Cerebral venous blood flow was estimated by a catheter introduced cephalad in the jugular vein. In arterial and venous blood we determined the haematocrit value; total serum protein, using PHILLIPS and VAN SLYKE's method; oxygen and haemoglobin contents, with the aid of an Atlas-type haematometer; the level of glucose, with the method of DURHAM et al. [3] slightly modified by us [31]; and the amount of pyruvic acid, according to FRIEDMANN and HAUGEN [7].

When blood pressure dropped to 60 mm Hg an infusion of noradrenaline was started, its rate was adjusted to maintain blood pressure between 100 and 120 mm Hg.

Observations were made and the data analyzed on five occasions during the experiment, *viz.* (1) at the end of the 4-hour ischaemia, before removing the tourniquet (control); (2) 15 minutes after its removal; (3) when blood pressure had fallen to 60 mm Hg; (4) after the first 15 minutes of noradrenaline infusion; (5) after the infusion, when blood pressure had become constant. In Tables II and III the results are summarized accordingly, and the columns in them numbered 1 through 5.

Results

In earlier experiments [32] it was shown that the survival of ischaemic shock was not prolonged by noradrenaline infused during the period of hypotension at an arterial pressure of 60 mm Hg. In the present experiments noradrenaline was infused until death had ensued. For the maintenance of blood pressure increasing doses were usually required; tachyphylaxis against noradrenaline appeared, similarly as in haemorrhagic shock [6]. Haemoconcentration and a fall in blood pressure occurred 15 to 30 minutes after removal of the tourniquet (Table I).

Cerebral circulation. Mean blood pressure, which was 137 mm Hg before the removal of the tourniquet, dropped to 89 mm Hg in 15 minutes after its removal. A decrease from 58.6 to 46.8 ml/min of the carotid flow was observed during the same time. Its changes varied parallel with those in blood pressure; it was 28.5 ml/min when the latter dropped to 60 mm Hg (Table II). Correspondingly, in the present experiments no significant changes were noted in peripheral resistance; a slight fall from 2.76 to 2.44 was registered after removing the tourniquet. Owing to the wide scattering this was not significant statistically. On the other hand, when blood pressure fell to 60 mm Hg, carotid resistance increased to 2.93 on the average, but this difference was not significant, either.

Table I

Haematocrit reading, total serum proteins, and survival after 4-hour ischaemia of the limbs

			During ischaemia	After release	After 1 hr	After 2 hrs	At death
	Haemat.	$\bar{x}$	45.0	58.2	60.1	57.4	60.7
Contr.		s.d.	11.7	6.4	8.1	7.3	8.6
n = 10	Prot.	$\bar{x}$	6.74	7.70	8.05	7.51	7.56
		s.d.	1.20	1.18	1.22	1.05	1.20
	Haemat.	$\bar{x}$	47.8	54.8	61.6	61.4	59.0
Noradr.		s.d.	10.3	6.5	7.8	6.9	6.9
n = 10	Prot.	$\bar{x}$	7.09	7.77	8.32	7.80	7.89
		s.d.	0.57	0.43	0.78	0.90	1.33

Survival

Contr.	$\bar{x}$	4h 07′	
	s.d.	3.55	t = 0.836
Noradr.	$\bar{x}$	2h 54′	p > 40%
	s.d.	1.53	

In the first 15 minutes the noradrenaline infusion induced a rise in blood pressure to 113.7 mm Hg, accompanied by an increase in carotid flow to 39.3 ml/min. As, however, the rise in carotid flow was not proportional to that in blood pressure, carotid resistance increased significantly, to 4.23. With the noradrenaline infusion completed, blood pressure immediately dropped to and became stable at a lower than the pre-infusion level (43.2 mm Hg). Carotid flow and resistance decreased approximately to the pre-infusion levels.

Cerebral metabolism. Oxygen consumption showed no change after removal of the tourniquet, obviously because the arteriovenous (carotid artery — jugular vein) oxygen difference increased with the decreasing carotid flow. The effect of the continued decrease in blood pressure and carotid flow could no longer be balanced by an increasing rate of oxygen consumption; consequently, when arterial pressure fell to 60 mm Hg, cerebral oxygen consumption was found to have diminished significantly while the arteriovenous O_2 difference rose to 12.4 vol.%. Upon the action of noradrenaline, oxygen utilization by the brain was found to have returned to almost the control value.

Consumption of glucose essentially paralleled that of oxygen. Each of the three pre-infusion analyses showed that 1.3 ml of glucose were taken up by the brain for every ml of oxygen consumed. After 15 minutes of noradrenaline infusion this relation changed slightly because the rate of glucose uptake exceeded that of oxygen consumption; 1.65 mg of glucose were utilized by the brain for every ml of oxygen. With the infusion completed, the rate of glucose uptake

Table II

		1	2	3	4	5
Blood press. mm Hg	$\bar{x}$	137.0	89.1	59.6*	113.7	43.2
n = 15	s.d.	28.9	9.4	7.9	18.6	15.1
Carot. flow ml/min.	$\bar{x}$	58.6	46.8	28.5*	39.3 !	28.3
n = 15	s.d.	27.7	21.0	17.0	23.9	28.3
Carot. resist. (PRE)	$\bar{x}$	2.76	2.44	2.93	4.23*	3.03
n = 15	s.d.	1.10	1.73	2.00	3.41	2.09
A—V O_2 diff. vol.%	$\bar{x}$	7.77	10.60	12.41*	9.60 !	11.83 !
n = 14	s.d.	3.45	4.49	2.02	2.57	3.53
Utiliz. of O_2 ml/min.	$\bar{x}$	4.78	4.94	3.64*	4.41 !	3.82
n = 14	s.d.	2.18	2.59	2.75	3.45	3.74
Utiliz. of gluc. mg/min.	$\bar{x}$	6.25	6.35	4.45	7.39 !	5.27
n = 13	s.d.	5.54	4.74	4.00	5.81	5.56
Utiliz. of pyruv. mg/min.	$\bar{x}$	0.29	0.33	0.08**	0.10**	0.07**
	s.d.	0.27	0.22	0.07	0.12	0.07

* = significant difference from control value at the 1 to 5 per cent level
** = significant difference from control value at the 0.1 per cent level
! = significant difference from preceding period at the 1 to 5 per cent level

decreased, and the brain again utilized about as much (1.38 mg) glucose per ml of oxygen as before the infusion.

In most of our experiments the brain was observed to take up pyruvic acid, as the pyruvic acid content was lower in the jugular vein than in the carotid artery. The calculated rate of pyruvic acid disappearance in the control period, and after the removal of the tourniquet, was 0.3 mg/min on the average. When blood pressure fell to 60 mm Hg, and cerebral oxygen and glucose utilization diminished, the rate dropped to 0.08 mg/min. The infusion of noradrenaline left it essentially unaltered notwithstanding the concurrent substantial increase in glucose utilization. During both the hypotensive state and the subsequent 15 minutes of noradrenaline infusion in the course of which blood pressure was normal, a loss of pyruvic acid was observed in 3 of the 13 cases, the pyruvate concentration having been higher in the venous than in the arterial blood.

Limb circulation. Removal of the tourniquet caused an immediate reduction of about 50 per cent in the arterial blood flow of the forelegs, and an in-

crease in resistance. The continued fall in blood pressure was paralleled by a fall in limb circulation. Although it brought blood pressure back to normal, noradrenaline did not influence limb circulation; the peripheral resistance, accordingly, increased to about double the control value. This showed that the vessels of the limbs had retained their reactivity to noradrenaline. The data presented in Table III are really no adequate expression of the true conditions, for in several experiments noradrenaline was found to have completely arrested the circulation in the arteries and the bubble flowmeter. As this would have meant the taking into account of a limitless increase in resistance, the results of these experiments had to be disregarded in calculating mean resistance and blood flow. Consequently, in Table III the values for blood flow are higher, and those for resistance, lower, than were the corresponding actual values.

Table III

Blood flow and resistance in brachial artery

		1	2	3	4	5
Blood flow ml/min.	$\bar{x}$	9.4	4.9**	3.5**	4.0**	1.6**
n = 12	s.d.	3.05	3.45	2.00	4.29	1.95
Resist. (PRE)	$\bar{x}$	14.06	18.2	16.1	28.5*	26.8

* = significant difference from control value ($p < 5\%$)
** = significant difference from control value ($p < 0.1\%$)

Discussion

On the evidence of our results, while capable of maintaining blood pressure in experimental ischaemic shock, noradrenaline is without influence on survival time. Similar observations have been made by other workers [6, 8] in haemorrhagic shock; only FOZZARD and GILMORE [5] claim to have achieved a significant prolongation of survival time by administering noradrenaline after the retransfusion of the withdrawn blood. The changes observed by us in blood pressure, haematocrit, and serum protein value were on the whole in agreement with those reported in the literature as obtained with similar methods [19, 30, etc.].

In our experiments cerebral blood flow decreased practically parallel with blood pressure. Until quite recently it was generally believed that the cerebral vessels were unable to control blood flow by vasomotion and that the latter passively followed the changes in arterial pressure [11, 34]. It has now been found by NOELL [21, 22] that cerebral blood flow followed pressure within the

normal limits only, and that after a certain critically low limit (70 to 90 mm Hg) has been reached, oxygen deficiency caused dilatation of the cerebral vessels. In man, on the other hand, a moderate fall in cardiac output and blood pressure has been shown to affect cerebral circulation slightly or not at all [2, 9, 10, 15, 25, 27]. Cerebral blood flow has been found to decrease only when arterial pressure fell by as much as 50 per cent, and even then it decreased at a comparatively low rate, indicating a decrease in cerebrovascular resistance [4, 27]. A decrease in vascular resistance has also been observed in dogs with haemorrhagic shock [6]. However, KOVÁCH et al. [17] noted passing cerebral vasodilatation after retransfusion only; without retransfusion blood flow through the dog brain changed parallel to blood pressure. In experimental ischaemic shock the same authors [26, 16] observed vasodilatation in the brain 2 to 10 minutes after removing the tourniquet; subsequently cerebral resistance remained at the control level. Accordingly, unlike in man, in the dog, hypotension caused by shock is not accompanied by a dilatation of the cerebral blood vessels. We have observed no decrease in resistance even at levels below 70 mm Hg. This may have been due to the haemoconcentration, which is known to increase the viscosity of blood and also vascular resistance in the brain [20, 22].

Cerebral oxygen consumption only decreased at very low blood pressure levels. In mild shock, the decrease in blood flow was balanced by an increase in cerebral arteriovenous O_2 difference. These findings agree well with earlier observations [4, 15, 23, 24, 27, etc.].

There was a certain parallelism between cerebral glucose uptake and oxygen consumption. With the exception of one case, after 15 minutes of noradrenaline infusion 1.3 mg of glucose were taken up for 1 ml of oxygen, exactly the amount which according to theoretical calculations and earlier experiments is necessary for glucose oxidation [12]. In man, cerebral glucose uptake amounts to 1.6 mg per 1 ml of oxygen; the slight difference is believed to be due to the conversion of glucose into pyruvic and lactic acid [14, 18]. The difference in cerebral glucose consumption between man and dog might be the explanation for the fact that whereas the human brain is known to lose pyruvic acid [13, 14], in the dog there was a notable uptake of pyruvate. Pyruvate was lost only during extreme hypotension.

Noradrenaline restored to normal the blood pressure in our experiments and increased carotid flow significantly, though not in proportion to pressure. Cerebrovascular resistance increased accordingly. In normal humans, by raising the blood pressure noradrenaline tends to reduce cerebral blood flow, in other words, it increases cerebrovascular resistance. FRANK et al. [6] observed in the dog with haemorrhagic shock that noradrenaline, apart from maintaining blood pressure, brought about an increase of blood flow in the sagittal sinus. In contrast with other workers, the quoted authors observed a marked cerebral vasodilatation during the period of hypotension.

Cerebral oxygen consumption increased with the increase in blood flow to nearly the control values. Glucose uptake increased simultaneously and considerably, while pyruvate uptake did not increase. In shock, this phenomenon might be due to the blockage postulated to exist between the glycolytic and tricarboxylic-acid cycles [28, 29], resulting in an accumulation of extracellular lactate and pyruvate. Despite improving circulation and cerebral oxygen supply, noradrenaline is thought to have no influence on that accumulation.

After the end of noradrenaline infusion, blood pressure abruptly dropped below the pre-infusion level, and cerebral blood flow and oxygen consumption decreased. The animals were in the state of severe shock, and most of them died in 30 to 60 minutes.

The changes in limb circulation and resistance were of the same direction as those in cerebral circulation. This makes it clear that the blood flow changes in the muscles and skin of the head could neither obscure nor distort the phenomena caused by changes in cerebral circulation.

The conclusion which seems to follow from the present experiments is that noradrenaline maintains blood pressure and increases cerebral blood flow, but not lineally; in other words, cephalic vascular resistance increases. There are signs indicating that notwithstanding its promoting oxygen consumption and glucose uptake by the brain, noradrenaline probably fails to counteract the disturbances of cerebral metabolism during shock.

REFERENCES

1. Catchpole, B. N., Hackel, D. B., Simeone, F. A.: Ann. Surg. **142,** 372 (1955).
2. Dewar, H. A., Owen, S. G., Jenkins, A. R.: Lancet **264,** 867 (1953).
3. Durham, W. F., Bloom, W. L., Lewis, G. T., Mandel, E. E.: Publ. Hlth Rep. **65,** 676 (1950).
4. Finnerty, F. F. Jr., Witkin, L., Fazekas, J. F.: J. clin. Invest. **33,** 1227 (1954).
5. Fozzard, H. A., Gilmore, J. P.: Naval Field Res. lab. Proj. No NM 71 06 09.1.6.
6. Frank, E. D., Frank, H. A., Jacob, S., Weizel, H. A. E., Korman, H., Fine, J.: Amer. J. Physiol. **186,** 74 (1956).
7. Friedman, T. E., Haugen, G. E.: J. biol. Chem. **147,** 415 (1943).
8. Gilmore, J. P.: Amer. J. Physiol. **195,** 473 (1958).
9. Hafkenschiel, J. H., Crumpton, C. V., Meyer, J. H., Jeffers, W. A.: J. clin. Invest. **29,** 403 (1950).
10. Hafkenschiel, J. H., Friedland, Z. K., Zintel, H.: J. clin. Invest. **33,** 57 (1954).
11. Hill, L.: The physiology and pathology of the cerebral circulation: An experimental research. Churchill, London, 1896.
12. Himwich, H. E.: Brain metabolism and cerebral disorders. Williams & Wilkins, Baltimore 1951.
13. Himwich, W. A., Himwich, H. E.: J. Neurophysiol. **9,** 133 (1946).
14. Kety, S. S.: in The metabolism of the nervous system, ed. D. Richter. Pergamon Press, London 1957.
15. Kety, S. S., King, B. D., Horvath, S. M., Jeffers, W. A., Hafkenschiel, J. H.: J. clin. Invest. **29,** 402 (1950).
16. Kovách, A. G. B., Roheim, P. S., Irányi, M., Cserháti, E., Gosztonyi, G., Kovách, E.: Acta physiol. Acad. Sci. hung. **15,** 217 (1959).
17. Kovách, A. G. B., Roheim, P. S., Irányi, M., Kiss, S., Antal, J.: Acta physiol. Acad. Sci. hung. **14,** 231 (1954).
18. Lassen, N. A.: Physiol. Rev. **39,** 183 (1959).

19. Mylen, E., Winternitz, M. C., Katzenstein, R., Sütő-Nagy, G. J.: Amer. J. Physiol. **137,** 280 (1942).
20. Nelson, D., Fazekas, J. F.: Arch. intern. Med. **98,** 328 (1956).
21. Noell, W.: Pflügers Arch. ges. Physiol. **247,** 528 (1944).
22. Noell, W.: Pflügers Arch. ges. Physiol. **247,** 553 (1944).
23. Noell, W., Schneider, M.: Pflügers Arch. ges. Physiol. **247,** 514 (1944).
24. Patterson, J. L. Jr., Cannon, J. L.: J. clin. Invest. **30,** 664P (1951).
25. Patterson, J. L. Jr., Warren, J. V.: J. clin. Invest. **31,** 653P (1952).
26. Roheim, P. S., Kovách, A. G. B., Irányi, M., Kovách, E., Cserháti, E., Gosztonyi, G.: Acta physiol. Acad. Sci. hung. **6,** Suppl. 26 (1954).
27. Scheinberg, P., Stead, E. A. Jr.: J. clin. Invest. **28,** 1163 (1949).
28. Simeone, F. A.: Fed. Proc. **20,** Suppl. 9, 3 (1961).
29. Stoner, H. B., Threfall, C. J.: in: The biochemical response to injury. Oxford, Blackwell 1960.
30. Swingle, W. W., Remington, J. W., Kleinberg, W., Drill, V. A., Eversole, W. J.: Amer. J. Physiol. **138,** 156 (1943).
31. Szabó, Gy.: Orv. Hetil. 912 (1956).
32. Szabó, Gy., Fekete, Gy., Renner, M.: unpublished.
33. Szabó, Gy., Magyar, Zs.: In press.
34. Wolff, H. G.: Physiol. Rev. **16,** 545 (1936).

Dr. György Szabó, Budapest, VIII, Mező I. út 15—17, Hungary

STUDIES ON ADAPTIVE ENZYME SYNTHESIS

I. GENERAL INTRODUCTORY REMARKS, AND METHODS

By

S. DÁN, L. ANTAL, GY. SZEGEDI and Á. GYÖRFFY

FIRST DEPARTMENT OF MEDICINE (DIRECTOR: PROF. B. FORNET) AND SECOND DEPARTMENT OF MEDICINE (DIRECTOR: PROF. GY. PETRÁNYI), UNIVERSITY MEDICAL SCHOOL, DEBRECEN

(Received September 22, 1962)

Carbon tetrachloride was found to reduce the tryptophan-pyrrolase content in the liver of the rat, and to impair the adaptive synthesis of this enzyme.

An indirect, bloodless, method has been elaborated for studying enzymic adaptation in the rat by the application of two consecutive tryptophan loads. The inductive first dose increases the amount of tryptophan pyrrolase in the liver, whereupon the second raises the production and urinary excretion of kynurenine and anthranilic-acid derivatives.

Double tryptophan loads administered in combination with histidine and cortisone, respectively, have confirmed these findings.

At the dawn of the history of adaptive enzyme synthesis, often termed enzymic adaptation, we find the work of WORTMANN [57]. He was the first to realize that amylase is produced by certain bacterial strains only when they have been growing in the presence of starch in the culture medium, and that in media not containing starch they gradually lose their ability to synthesize that enzyme. This means that certain bacterial strains are capable of altering their enzymatic composition, that is, of adapting it to alterations in the quality of the food available to them. Recent authors suggest the term enzyme induction [RICHTERICH, 50] for the phenomenon.

Adaptive enzyme synthesis has for long been known to occur in vertebrates. In the dog, the appearance of much lactase was demonstrated in the pancreas after orally administered lactose, and of invertase in the blood plasma following injection of sucrose [WEINLAND, 55]. No amylase could be shown to be present in the saliva of the dog unless it had been fed carbohydrates [NEILSON and TERRY, 43]. Old and recent observations alike provide evidence that the saliva of humans living on food rich in carbohydrates contains two or three times as much amylase as that of people living on a high-protein diet poor in carbohydrates [SIMON, 51; SQUIRES, 52].

Bacteriologists began to study adaptive enzyme synthesis systematically as early as the '30s and soon realized its practical importance (resistance to antibiotics). Pertaining research in vertebrates is less advanced. The early work on it was assessed in a monograph by KNOX, AUERBACH and LIN [30]. Their consistently grouped data convincingly demonstrate the important part

played by enzymic adaptation in the quantitative and qualitative changes of intracellular and extracellular enzymes due to the effect of various endogenous and exogenous factors, such as development, sex, neurohormonal regulation, the removal of endocrine glands, the administration of hormones, the quality of food, etc. As one of the manifestations (readily determined with biochemical methods) of the organism's broad adaptive capacity, adaptive enzyme synthesis is obviously an essential link in the chain of mechanisms controlling metabolic and energy processes.

Investigations into the vertebrates' ability to synthesize adaptive enzymes are beginning to unfold themselves under the pressure of current pharmacodynamic problems, but the need of investigations into the related pathophysiological and clinical questions does not yet seem to make itself widely felt. We ourselves have become aware of it in the course of earlier studies concerned with amino acid metabolism in patients with liver disease. Those studies resulted in the conclusion that the causes of disorders of amino acid metabolism in hepatic disease should be looked for in the difference between the normal and the diseased liver's biochemical adaptive capacity; in other words, the ability to synthesize adaptive enzymes [DÁN, 11, 12]. This conclusion was the stimulus for the present study of changes in adaptive enzyme synthesis under pathological conditions, and their metabolic consequences, on the one hand, and of possible ways and means to utilize our pertaining knowledge for diagnostic purposes, on the other hand. A number of questions arose. In what way do diseases influence the ability of the organism and of individual organs to synthesize adaptive enzymes? Do pathologic conditions impair the mechanism of adaptive enzyme synthesis? Do disorders of adaptive enzyme synthesis (its absence, insufficient or excessive degree) affect the changes associated with pathological processes?

An investigation into the adaptive changes of tryptophan pyrrolase (TPO) seemed to be a rewarding initial step of the experiments, and this on more than one account. First, the adaptive synthesis of TPO is a rapid process, easy to induce, and readily reproducible; the factors governing its time course [34], specificity, and relations to the neurohormonal system [28], are known. These facts would establish a basis on which to evaluate data obtained in pathological conditions. Secondly, the part played by an impaired ability of adaptive enzyme synthesis in the disorders of tryptophan metabolism, which are observable in various diseases, still await clarification. Thirdly, up to the present the method of studying the adaptive changes in animals, involved direct determination of the TPO content in liver homogenates. We have now elaborated a simple, indirect, bloodless method; it is based on the effect of a double tryptophan load on the quantity and quality of tryptophan metabolites excreted with the urine. A procedure of this type might make it possible to study adaptive enzyme synthesis for its use for diagnostic purposes.

In the present report we propose to deal in detail with the indirect method referred to above. Nevertheless, it seems necessary briefly to discuss first one of our earlier model experiments [12] concerning the disturbances of adaptive enzyme synthesis, which had been carried out with a direct (liver homogenate) method.

Adaptive synthesis of tryptophan pyrrolase in acute experimental hepatic disease

TPO occurs in large amounts in the liver. Its function — the oxidative splitting of the indole ring in tryptophan — was first observed by KOTAKE, ITAGAKI and NAKAYAMA [33], and studied in detail by BRAUNSTEIN [5] and KNOX [29]. The technique for studying the adaptive changes of TPO in animal experiments was developed by KNOX et al. [31]. Intraperitoneally, orally, or subcutaneously administered dl-tryptophan was found to increase to a manifold the TPO content of the liver of the rabbit, the rat, and the guinea pig. Experiments with labelled amino acids [18] and with ethionine, which inhibits adaptive TPO synthesis, produced evidence of an increased enzyme synthesis, and no rise in enzyme activity [49, 35].

Experimental

Male rats weighing between 180 and 230 g, were used. Acute liver damage was produced with carbon tetrachloride; three doses of 0.2 ml per 100 g body weight were administered at two-day intervals. The prescriptions of KNOX [31] were followed in performing the enzyme induction experiments.

The experimental animals were injected intraperitoneally with 0.3 g of sterilized dl-tryptophane suspended in 3 ml of physiological saline; the controls received 0.3 ml of sodium chloride, by the same route. Five hours later, all the animals were decapitated and exsanguinated, the livers promptly removed, and precisely weighed portions of each homogenized at 0 to 4° C, with quartz sand or powdered glass and 1.18 per cent potassium chloride 1 : 10. Following centrifugation for half a minute, the homogenates were used immediately. The incubation mixture contained 2 ml of homogenate, 0.5 ml of 0.2 *M* phosphate buffer adjusted to pH 7, and 0.3 ml of 0.03 *M* l-tryptophan. The reactions were run in open flasks in a water bath at 38° C with constant shaking for one hour. Deproteinization was carried out with 2 ml of 5 per cent zinc acetate and 2 ml of 0.18 n NaOH. In the corresponding blanks, tryptophan was added at the end of the incubation period, immediately before deproteinization. After standing for half an hour, the reaction mixtures were filtered, and the kynurenine in the filtrates was estimated in 10 mm layer thickness, in a Beckman spectrophotometer, at 360 mμ. 0.1 *M* kynurenine/ml = 0.440 extinction value.

For the sake of brevity, in the following the adjectives "spontaneous" and "induced" will be used to denote, and distinguish between, animal groups not treated and treated with tryptophan, respectively.

Results

These are shown in Fig. 1, which illustrates the following:

I. Group of normal animals (A)

The results obtained confirm the laws governing adaptive enzyme synthesis as established by KNOX.

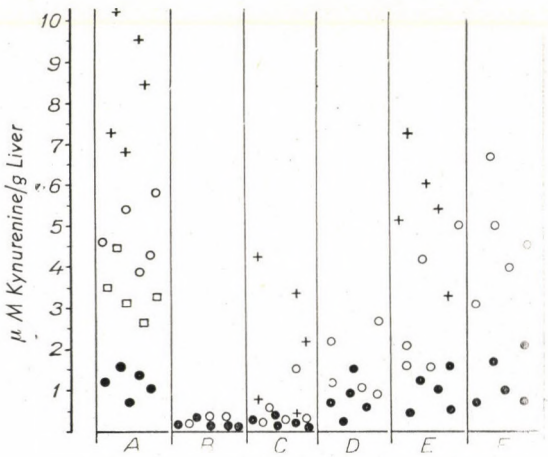


Fig. 1. Spontaneous and induced TPO contents of the liver of intact rats and rats treated with carbon tetrachloride. Enzyme activity expressed in μmoles of kynurenine per g of liver per hour. Column A: group of intact animals; columns B—F: groups of CCl_4 treated animals. Time of induction: 1 day after the last day of CCl_4 treatment for group B; two days after it for C; four for D; seven for E; and ten for F. Symbols: • = spontaneous TPO content; ○ = TPO content induced with tryptophan; + = with combined tryptophan and cortisone; □ = with histidine

a) The TPO content of the liver was found to be substantially greater in the tryptophan-treated than in the untreated animals. The difference in hepatic TPO content between the spontaneous and induced groups expresses the degree of adaptive enzyme synthesis. Though there were individual differences within the groups, the difference between the spontaneous and induced values was significant.

b) Combined administration of cortisone and tryptophan increased adaptive enzyme synthesis.

c) Histidine treatment induced the adaptive synthesis of TPO.

II. Groups of CCl_4 treated animals (B to F)

a) During the first 48 hours of CCl_4 treatment (groups B and C), the spontaneous TPO level was low in the liver of all the animals, and no adaptive enzyme synthesis could be induced with tryptophan.

b) Beginning with the fourth day of treatment (group D), marked individual differences were displayed by both the spontaneous and the induced animal groups; though considerable induction was observed, there were animals with TPO levels similar to those in groups A and B.

c) Still more marked were the individual differences within the groups on the seventh and tenth day (groups E and F).

d) To the combined administration of cortisone and tryptophan, the animal were found to react with considerable adaptive enzyme synthesis in those groups in which tryptophane alone was of no (group C) or of only slight effect (group E).

The wide differences observed, even under identical experimental conditions, in the CCl_4 treated animals were obviously due to differences from one animal to the other in the nature and extent of the parenchymal and biochemical damage.

We have no knowledge of the changes which adaptive enzyme synthesis undergoes in the successive phases of recovery, and of whether recovery can be influenced pharmacologically. It is possible to study these points by means of the liver homogenate method in large animal groups, but to obtain close knowledge of the nature of the biochemical damage, it appears desirable repeatedly to study adaptive enzyme synthesis in the same animals in each of the consecutive phases. A procedure of this type might be the basis of studies of the adaptive enzyme synthesis for diagnostic purposes.

Adaptive synthesis of tryptophan pyrrolase following two successive tryptophan loads

The adult organism needs daily 7 mg per kg body weight of tryptophan. In the normal organism, most of it is used for protein synthesis, 1 per cent for the production of serotonin, and 1 to 2 per cent for that of nicotinic acid. The proportion of tryptophan used up by intestinal bacteria is not known with certainty. TPO has a role to play in the first step of tryptophan-nicotinic acid metabolism (Fig. 2).

Clinical observations and animal experiments supply evidence that tryptophan-nicotinic acid metabolites may be present in the urine of normal individuals [3, 7, 9, 16, 17, 19]. Their quantity and quality can be influenced by the administration and depletion of B vitamins [6, 7, 10, 15], large doses of tryptophan [21, 22, 23, 24, 25, 39, 45], and the quality of the food ingested; in

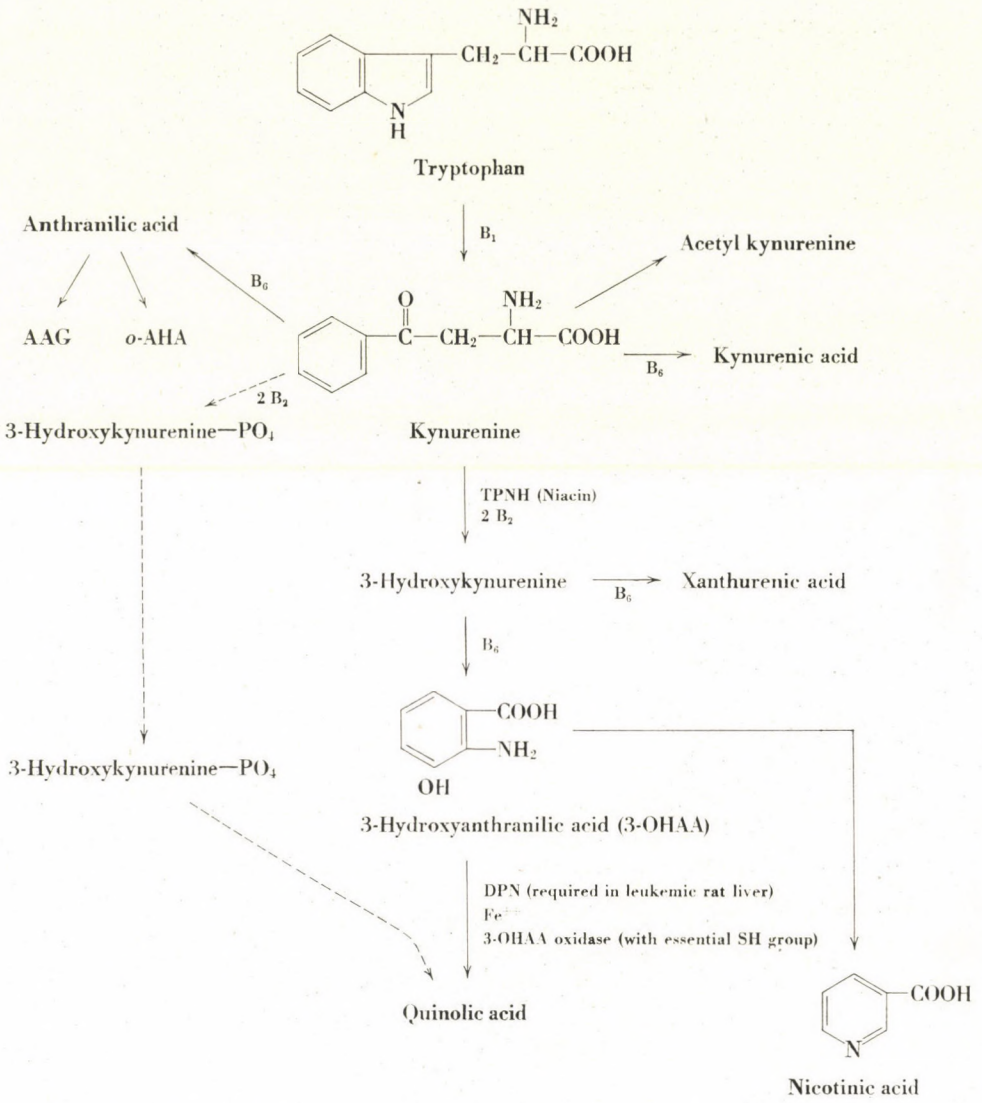


Fig. 2. Outline of tryptophan-nicotinic acid metabolism (MARVER, H. S.: J. Lab. clin. Med. *58*. 425 [1961])

addition, they may be deficient or excessive in amount in a variety of diseases [1, 4, 8, 10, 14, 19, 36, 42, 45, 46, 47, 48, 53, 54]. Data of this kind are suggestive of a disturbance of the tryptophan-nicotinic acid metabolism, but do not yield information about the very step of adaptive enzyme synthesis responsible for the development of the metabolic defect.

A double tryptophan load seemed to be a promising method for investigating this open question. There were obvious reasons to suggest that if the

first dose of tryptophan induced adaptive synthesis of TPO, the second would produce the more urinary kynurenine and other metabolites, the more effective the induction had been.

Methods

Rats of about the same age, weighing between 180 and 230 g, were fasted for 16 hours but were allowed to drink water. For the time of the experiment each animal was kept in a wide funnel, built into a support and covered with wire netting, or in a funnel-shaped glass vessel, with the tube at its point inserted into a flask put under it to collect the urine.

By a stomach tube, 7 ml of a tryptophan solution, prepared as described below, were introduced at 0 hour; at 2 hours this was followed by 6 ml of tap water; at 4 hours, by a second dose of tryptophan; and at 6 hours by further 6 ml of tap water. Urine specimens were collected at 2-hour intervals, and denoted I, II, III and IV, respectively. At the end of each collection period the animal was lifted a little, but not taken out of the funnel or vessel, and the skin over the abdomen was gently pinched to cause the bladder to empty. Nor was the animal lifted beyond the brim of the funnel or vessel, while the tube was introduced into the stomach, for experience showed that it frequently passed urine during these manoeuvers. Collected under these conditions, fraction I varied in amount from 1 to 3 ml; fractions II and III each from 2 to 6 ml, and fraction IV from 3 to 5 ml, of urine. Confinement to funnel or vessel, introducing the stomach tube, and the tryptophan, were well tolerated by the animals.

The urine fraction were made up to 30 ml with distilled water. The metabolite determinations were carried out at the end of the 8-hour experiment. Preparations for chromatography were begun the following day; meanwhile the urine was stored in the refrigerator.

The tryptophan solution was prepared on the day of the experiment; 7 ml of it contained the single dose of tryptophan. When, for instance, four rats were to be given each two successive doses of 400 μM tryptophan, then 4000 μM tryptophan were measured and dissolved by dropwise addition of 1 to 1.3 ml of 5 per cent sodium hydroxide. This alkaline solution was diluted with tap water to about 50 ml, and the pH adjusted to between 7 and 7.5 with 5 per cent hydrochloric acid, and made up to a final volume of 56 ml with tap water.

For paper chromatography the urine specimens were prepared according to HARTMANN's description. Schleicher—Schüll filter paper No. 2043b was used. The solvent systems were isopropanol : ammonia : water (200 : 10 : 20) and butanol : water : acetic acid (120 : 150 : 30). The chromatograms were developed with para-dimethylamino-benzaldehyde (DBA) in 10 per cent hydrochloric acid, diluted 1 : 4 with acetone immediately before development. All chromatograms were also studied under ultra-violet light.

Kynurenine and the metabolites reacting with DBA were determined with the slightly modified method of OTANI, NISHINO and IMAI [44], respectively WISS and HATZ [56]. The underlying principle is that with DBA many tryptophan metabolites form coloured compounds. The russet colour corresponding to kynurenine can be extracted with butanol. The intensity of the violet colour of the remaining aqueous solution depends on the amount of other metabolites reacting with DBA. This amount is expressed as the number of μ moles of anthranilic acid corresponding to the colour intensity produced by all the compounds reacting with DBA. In the Figures and Tables this is denoted by *As*.

The following reagents were used:

(*a*) Two g of DBA dissolved in 30 per cent hydrochloric acid and made up with distilled water to 100 ml.

(*b*) Freshly prepared 3 per cent hydrogen peroxide.

(*c*) Ten per cent trichloro-acetic acid.

Three ml of diluted urine and 3 ml of trichloro-acetic acid were mixed by shaking in tubes, allowed to stand for 5 minutes, and centrifuged. Of the clear liquid, 4 ml amounts were measured into glass vessels or thin 8 to 10 ml centrifuge tubes. After addition of 0.3 ml of DBA and 0.15 ml of H_2O_2 the vessels or tubes were closed with a rubber stopper, turned upside down a few times to attain intimate mixing, and placed in a thermostat at 37°C for 15 hours. During this time a brown colour growing gradually darker was developing.

The coloured solution was shaken for 2 minutes with 3 ml of distilled butanol, and centrifuged. Using a Pasteur pipette, the butanolic phase was drawn off by suction, and the extraction repeated with another 3 ml of butanol. The two butanolic extracts were then pooled. On a few occasions both the aqueous and the butanolic extracts had turned turbid, but centrifugation for a minute or two cleared them sufficiently for photometry. In cuvettes of 10 and

5 mm the butanolic and aqueous solutions, respectively, were read in a Stufenphotometer with S 56 filter against a blank. This was prepared by adding the reagents to 4 ml of 5 per cent trichloro-acetic acid and, after standing at 37 °C for 15 hours, extraction with butanol.

The amount of kynurenine formed was calculated according to Otani's formula (kynurenine mg/ml = 0.370(Ext.—0.059).0.5. The amount of anthranilic acid corresponding to the extinction of the aqueous solution was read off the extinction curve. An aqueous stock solution was prepared containing 300 γ of anthranilic acid per 2 ml. Of this, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 and 1.0 ml amounts were measured into test tubes and made up to 2 ml with distilled water. Following the addition of 2 ml of trichloro-acetic acid and the colour reagents, the mixture was placed in the thermostat for 15 hours. Photometry with an S 56 filter followed in a 5 mm cuvette. In this manner, 0.01 Ext. = 1.38 γ of anthranilic acid per 2 ml.

Taking into account Otani's formula, the data yielded by the extinction curves, as also the facts that every urine fraction was diluted to 30 ml and that the determinations were made from 2 ml amounts of that dilution, the kynurenine and anthranilic acid contents of the individual fractions can be calculated according to the formulas,

μM kynurenine/30 ml = 33.3 (Ext. — 0.059),

μM anthranilic acid/30 ml = Ext. = Ext. 15.0.

Results and discussion

Figs 3 and 4 inform about the excretion of metabolites in the individual urine fractions.

The one-directional chromatogram was studied also under ultraviolet light. Fraction III was conspicuous for the intense fluorescence of kynurenine (Rf: 0.55, light blue) and 3-oxyanthranilic acid (Rf: 0. 90, bluish-violet).

Although only the six compounds named in the legend to Figs 3 and 4 could be identified with certainty, the chromatograms appear to permit the following statements.

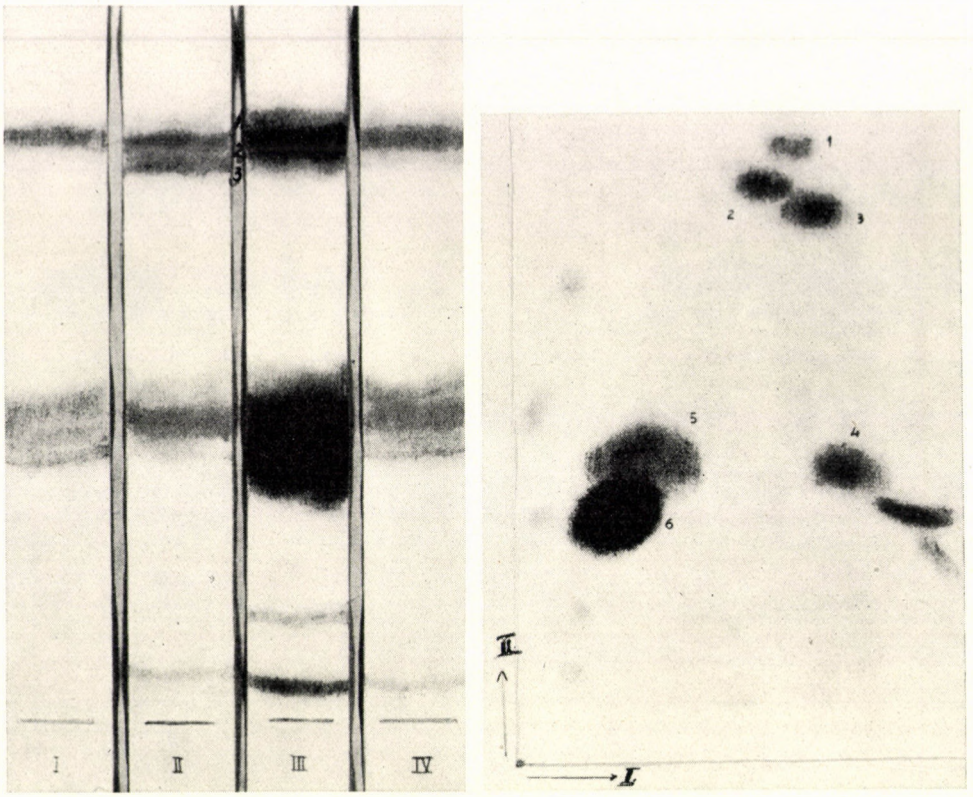
1. Urinary kynurenine excretion after the second tryptophan load (fraction III) considerably exceeds that after the first load (fraction I).

2. The other compounds reacting with DBA are likewise present in substantially higher amounts in fraction III than in either fraction I or II. This indicates that upon the effect of the first tryptophan load not only TPO but also other enzymes of the tryptophan-nicotinic acid multienzyme system have adapted themselves (simultaneous adaptive enzyme synthesis?).

3. Only future investigations can decide whether the excess metabolite excretion observed in fraction III was actually related to enzyme synthesis induced by the first tryptophan dose or whether it represented the sum total, manifesting itself in fraction III, of the urinary metabolite excretions caused by the joint effect of the first and second tryptophan loads.

Quantitative determination of the urinary kynurenine and other metabolites reacting with DBA, will answer this question.

In six groups, four animals at a time were subjected to double tryptophan loads. Figs 5 to 10 present the results obtained in the individual groups. The arabic numerals on the horizontal axis indicate the time course of the experiment. Arrows below the diagrams indicate the times at which tryptophan, tap water, cortisone, or histidine, was administered. White columns in the diagrams indicate urinary kynurenine excretion, and dark columns urinary anthranilic



Figs. 3 and *4*. One-dimensional (butanolic) chromatogram of fractions I, III, III, and IV, and two-dimensional chromatogram of fraction III, showing only the spots that have become sharply outlined during development. Based on the colour and the Rf value of the individual spots: 1 = anthranilic acid (yellow); 2 = hydroxyanthranilic acid (light-brown); 3 = acetyltryptophan? (bluish-violet); 4 = carbamide (yellow); 5 = tryptophan (green); 6 = kynurenine (orange-red)

acid excretion in the individual fractions, expressed in μmoles. At the bottom of the diagrams, the kynurenine, respectively the anthranilic acid, contents are shown in μmoles separately for fractions I + II and III + IV.

The diagrams show that the results have confirmed the chromatographic finding that urinary metabolite excretion after the second tryptophan load invariably and considerably exceeds that following the first load. The values for III + IV are all higher than those for I +II. The experiments described in the following, supply evidence in favour of a relationship between this excess and adaptive enzyme synthesis.

The first two diagrams in Figs 5, 7, and 10, and the first one in Fig. 9, illustrate experiments in which the animals received only a single dose of tryptophan (at zero hour) followed by tap water at 2, 4 and 6 hours. In these experiments fractions III and IV were found to contain little kynurenine and an-

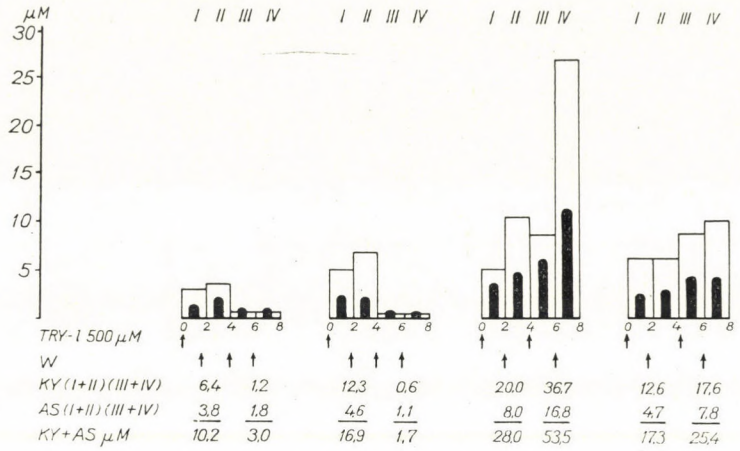


Fig. 5. Double tryptophan load, each with 500 μM of l-tryptophan. Try: tryptophan; As: amount of anthranilic-acid derivatives expressed as μM of anthranilic acid; W = tap water; ↑ : time of administration of water or amino acid

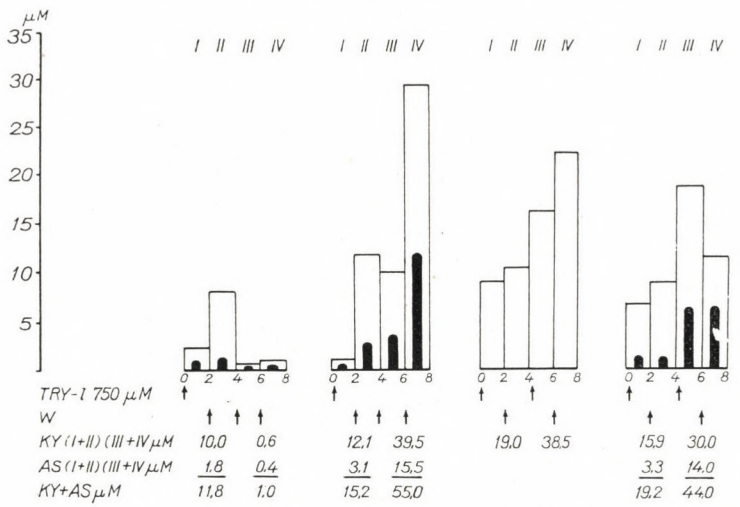


Fig. 6. Double tryptophan load, each with 700 μM of l-tryptophan

thranilic acid, particularly if viewed in relation to the same fractions in experiments with a double tryptophan load. Methodologically, this is an important point, for in double-load experiments it permits the kynurenine and anthranilic acid contents of fractions III and IV to be regarded as originating almost entirely from the second tryptophan dose. Accordingly, it seems justified to assume the kynurenine and anthranilic acid contents of fractions III and IV, which follow the second dose of tryptophan, to be directly related to the induced enzyme content.

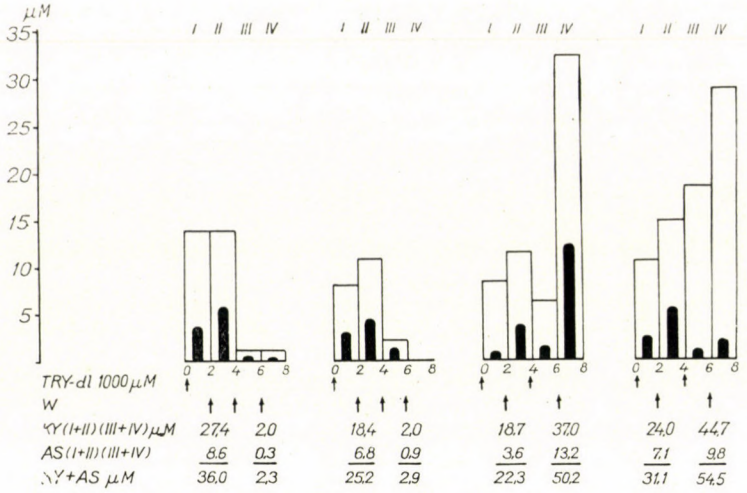


Fig. 7. Double tryptophan load, each with 1000 µM of l-tryptophan

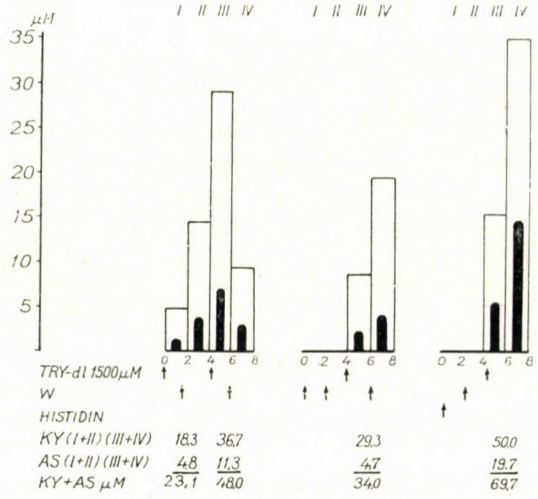


Fig. 8. Combined tryptophan and histidine treatment. First group of columns shows data of double tryptophan load with 1500 µM. In the other two experiments tryptophan was administered only once, at 4 hours, preceded by tap water (second group of columns), respectively by histidine (third group of columns)

In the experiment illustrated in the 3rd diagram of Fig. 8 the animal was given histidine instead of tryptophan at zero hour, and only one dose of tryptophan, at 4 hours. Neither kynurenine nor anthranilic acid could be shown to be present in fractions I and II, yet in fractions III and IV both were present in about the same amounts as after a double tryptophan load.

In the experiments illustrated in the 3rd and 4th diagrams of both Fig. 9 and Fig. 10 the animals were given at zero hour, simultaneously with oral tryp-

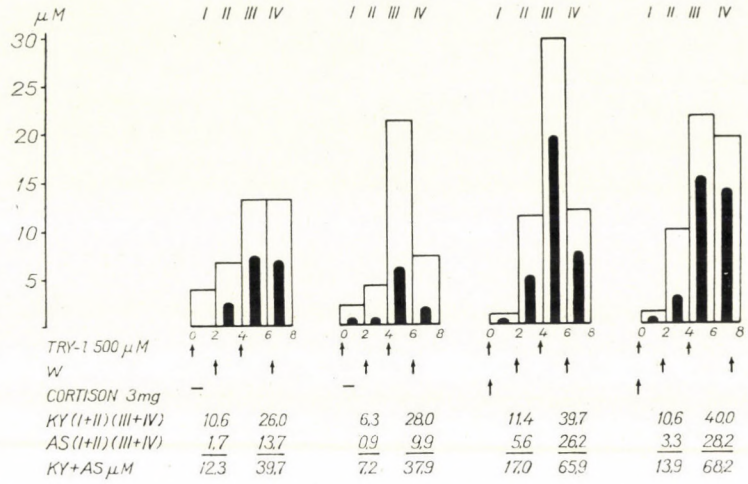


Fig. 9. Combined cortisone and double tryptophan load, with 500 μM of l-tryptophan

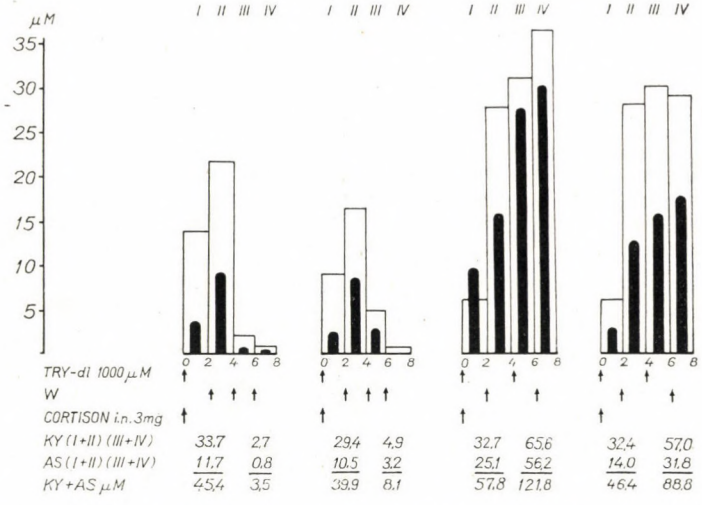


Fig. 10. Combined cortisone and double tryptophan load, with 1000 μM of dl-tryptophan

tophan, 3 mg of intraperitoneal hydrocortisone. The results obtained show that in these animals the kynurenine and anthranilic acid contents of fractions III and IV greatly exceeded those in the animals not treated with cortisone; further, that the anthranilic acid : kynurenine ratio was substantially higher in the former than in the latter.

The similarity apparent between the data in Figs. 8 and 10 and those obtained in the liver homogenate experiments, makes it seem obvious that a relation exists between the changes observed in the kynurenine and anthranilic

acid contents of fractions III and IV, and the ability of histidine to induce adaptive synthesis of TPO, respectively the ability of cortisone to intensify adaptive enzyme synthesis.

In liver homogenate experiments, adaptive enzyme synthesis can be measured by and expressed as the difference in the renal TPO content between the spontaneous and induced groups. Another question is as to whether there exists some quantitative relationship between urinary kynurenine and anthranilic acid excretion on the one hand, and adaptive enzyme synthesis on the other. Studies of the adaptive synthesis of TPO [34, 35] have shown that it starts in the liver within the first hour following the administration of the inducing tryptophan and, increasing evenly with time, it reaches its peak between the 5th and 8th hour. This knowledge entitles to the supposition that the spontaneous TPO content of the liver is most probably related to the urinary kynurenine and anthranilic acid excretion in fraction I, and the induced TPO content to the excretion in fraction III. This view is favoured by our finding that the kynurenine and anthranilic acid content of fraction III is always a manifold of that of fraction I, though, as the data in our figures show, not independently of the amount of tryptophan administered. It is of interest in this connection to note the changes in the ratio of anthranilic acid to kynurenine, particularly its increase in the experiments with cortisone. This increase presumably bears a relation not only to the adaptive increase of TPO, but also to that of the other members of the tryptophan-nicotinic acid enzyme system.

The tasks we set ourselves in this study were to elaborate a method for use in double tryptophan load experiments, to establish the adequate amount of tryptophan to be administered, and the best procedure for the collection of urine specimens; in addition, to select the method of kynurenine and anthranilic acid determination most suitable for routine work. The results of our investigations appear to confirm that the double-load method is suitable for assaying adaptive enzyme synthesis, since the first dose of tryptophan induces adaptive synthesis of TPO, *i. e.* increases the TPO content of the liver, whereupon the second dose gives rise to an elevated production and, accordingly, an elevated urinary excretion, of tryptophan metabolites.

It remains for subsequent experiments to supply answers to two more questions.

a) In what manner are the adaptive changes of the tryptophan-nicotinic acid metabolites influenced under normal and pathologic conditions, by the quality of food, the organism's tryptophan balance, and by endocrinologic and pharmacologic effects?

b) In what measure can the principle underlying the double tryptophan load method be extended for use in assaying the adaptive synthesis of other enzymes?

REFERENCES

1. ALTMANN, K. I., MILLER, G.: Nature (Lond.) **172,** 868 (1953).
2. AURICCHIO, S., QUAGLIARELLIO, E., RUBINO, A., VECCHIONE, L.: Ann. pediat. (Basel) **194,** 11 (1960).
3. BIEHL, P. J., VILTER, R. W.: Proc. Soc. exp. Biol. (N. Y.) **85,** 389 (1954).
4. BOYLAND, E., WILLIAMS, D. C.: Biochem. J. **64,** 578 (1956).
5. BRAUNSTEIN, cit.: KNOX. 28/a.
6. BROWN, R. R.: J. biol. Chem. **227,** 649 (1957).
7. BROWN, R. R., PRICE, J. M.: J. biol. Chem. **219,** 985 (1956).
8. CATHIE, I. A. B.: Arch. Dis. Childh. **25,** 313 (1950).
9. DALGLIESH, C. E.: Advanc. Protein Chem. **10,** 33—124. Academic Press, New York, 1955.
10. DALGLIESH, C. E., TEKMAN, S.: Biochem. J. **56,** 458 (1954).
11. DÁN, S.: Data to the theoretical and practical problems of functional diagnostics of liver. Dissertation, Budapest, 1956.
12. DÁN, S., ANTAL, L., GYÖRFFY, Á., BEREGSZÁSZI, J.: In press.
13. DÁN, S., HAPPICH, GY., VAJDA, I., PÁSZTOR, J.: Orv. Hetil. **94,** 973 (1953).
14. DIAMOND, L. K., BLACKFAN, K. D.: Amer. J. Dis. Child. **56,** 464 (1938).
15. GASSMANN, B. KNAPP, A.: Klin. Wschr. **36,** 189 (1959).
16. GLAZER, H. S., NUELLER, J. F., THOMPSON, C., HAWKINS, V. R.,VILTER, R. W.:Arch. Biochem. **33,** 243 (1951).
17. GREENBERG, I. D., BOHR, D. F., GRATH, H. M., RINEHART, J. F.: Arch. Biochem. **21,** 237 (1949).
18. GROS, P., TALVAR, G. P., COURSAGET, J.: Bull. Soc. Chim. biol. (Paris) **36,** 1569 (1954).
19. HARTMANN, F., FREIBERG, E., RUGE, W.: Klin. Wschr. **40,** 721 (1962).
20. KÄHLER, H., HEILMEYER, L.: Ergebn. inn. Med. Kinderheilk. **16,** 292 (1961).
21. KNAPP, A.: Nahrung **2,** 568 (1958).
22. KNAPP, A.: Dtsch. Gesundh.-Wes. **16,** 941 (1961).
23. KNAPP, A., GASSMANN, B.: Klin. Wschr. **36,** 732 (1958).
24. KNAPP, A., ZIMMERMANN, H., GASSMANN, B.: Klin. Wschr. **36,** 819 (1958).
25. KNAPP, A., KUPKE, G.: Z. ges. inn. Med. **14,** 810 (1959).
26. KNAPP, A., KUPKE, G., GASSMANN, B.: Ernährungsforsch. **3,** 546 (1958).
27. KNOX, W. E.: Brit. J. exp. Path. **32,** 462 (1951).
28. KNOX, W. E.: Proc. International Symposium on Enzyme Chemistry. Tokyo and Kyoto 1957, Pergamon Press, London.

28a. KNOX, W. E.: in Ciba Symposium on Adaptive Enzymes in Animals. ed., Churchill, London, 1961.

29. KNOX, W. E., AUERBACH, V. H.: J. biol. Chem. **214,** 307 (1955).
30. KNOX, W. E., AUERBACH, V. H., LIN, E. C. C.: Physiol. Rev. **36,** 164 (1956).
31. KNOX, W. E., MEHLER, A. H.: J. biol. Chem. **187,** 419 (1950).
32. KNOX, W. E., MEHLER, A. H.: Science **113,** 237 (1951).
33. KOTAKE, J., ITAGAKI, C., NAKAYAMA, J.: Hoppe-Seylers Z. physiol. Chem. **270,** 83 (1941).
34. LEE, N. D.: J. biol. Chem. **219,** 211 (1955).
35. LEE, N. D., WILLIAMS, R. H.: Biochim. biophys. Acta (Amst.) **9,** 698 (1952).
36. LESCHER, F. G., HUBBLE, D.: Quart. J. Med. **1,** 425 (1932).
37. LOJKIN, M. E.: Nutrition **59,** 443 (1956).
38. LOJKIN, M. E., WERTZ, A. V., DIETZ, CH. G.: J. Nutr. **46,** 335 (1952).
39. MARVER, H. S.: J. Lab. clin. Med. **58,** 3, 425. (1961).
40. MASON, M.: J. biol. Chem. **201,** 513 (1953).
41. MEHLER, A. H., MCDANIEL, E. G., HUNDLEY, J. M.: J. biol. Chem. **232,** 331 (1958).
42. MUSAJO, L., BENASSI, C. A., PARPAJOLA, A.: Clin. chim. acta **1,** 229 (1956).
43. NEILSON, C. H., TERRY, O. P.: Amer. J. Physiol. **15,** 406 (1905—1906) cit. RICHTERICH p. 81.
44. OTANI, S., NISHINO, N., IMAI, K.: Hoppe-Seylers Z. physiol. Chem. **270,** 60 (1941).
45. PLAZZA, M.: Nature (Lond.) **191,** 601 (1961).
46. PRICE, J. M.: Univ. Mich. med. Bull. **33,** 289 (1958).
47. PRICE, J. M., BROWN, R. R., FORDE, A.: Proc. Amer. Ass. Cancer Res. **2,** 140 (1956).
48. PRICE, J. M., BROWN, R. R., RUKAVINA, J. G., MENDELSON, C., JOHNSON, S. A. M.: J. invest. Derm. **33,** 289 (1957).
49. PRICE, J. B., DIETRICH, L. S.: J. biol. Chem. **227,** 633 (1957).
50. RICHTERICH, L.: Enzympathologie. Springer, Berlin 1958.
51. SIMON, L. G.: J. Physiol. Path. gén. **9,** 261 (1907), cit. RICHTERICH, p. 81.
52. SQUIRES, B.: J. Physiol. (Lond.) **119,** 153 (1953).

53. Vogel, P., Erf, L. A., Rosenthal, N.: Amer. J. clin. Path. **7**, 436 (1937).
54. Wachstein, M., Gnaditis, A.: J. Lab. clin. Med. **40**, 530 (1952).
55. Weinland, E.: Z. Biol. **40**, 386 (1900); **47**, 279 (1906).
56. Wiss, O., Hatz, F.: Helv. chim. Acta **32**, 532 (1949).
57. Wortmann, J.: Z. physiol. Chem. **6**, 287 (1882).

Dr. Sándor Dán, First Department of Medicine
Dr. Lajos Antal, Dr. Gyula Szegedi, Dr. Árpád Györffy, Second Department of Medicine, University Medical School, Debrecen, Hungary

STUDIES OF THE URINARY STEROIDS IN HIRSUTISM AND VIRILISM

By

M. JULESZ, I. FAREDIN, I. TÓTH, MARGIT A. DÁVID and K. KOVÁCS

with the technical assistance of I. Szabadi, I. Simor, I. Szabadai

FIRST DEPARTMENT OF MEDICINE, UNIVERSITY MEDICAL SCHOOL, SZEGED

(Received January 21, 1963)

On the basis of the clinical appearance and diagnostic examinations 49 patients with hirsutism were classified into four groups and in these the urinary excretion of neutral total 17-ketosteroids, 17-ketosteroid fractions (beta-17-ketosteroids, androsterone, etiocholanolone, 11-oxygenated-17-ketosteroids), total 17-hydroxycorticosteroids, pregnanediol and oestrogen fractions (oestrone, oestradiol-17 beta, oestriol) has been studied.

According to the results obtained, steroid assays make it possible to differentiate the hirsutisms of adrenocortical origin from the other types, but allow no further differentiation. Etiocholanolone excretion in these patients was increased, and that of oestrone and oestradiol, decreased. These findings have been discussed in the light of recent data in the literature and it is suggested that an alteration in the reactivity of peripheral tissues and cells may play a role in the pathogenesis of hirsutism.

Hirsutism represents a serious emotional problem for the patient and a difficult diagnosis with many disappointments for the endocrinologist. The disappointments are due to the fact that the basic cause of the condition mostly remains undetected.

Several forms of hirsutism have been described. The scarce evidence available in respect of aetiology does not justify fine distinctions. Some authors only separate hirsutism from virilism (BISHOP, 1954; DORFMAN et al. 1956). Hirsutism is understood to mean an excessive growth of hairs and eventually with baldness of the masculine type, without symptoms of defeminisation. Virilism, on the other hand, means a condition where symptoms of defeminisation are associated to those of masculinisation. Defeminisation means amenorrhoea, diminution of libido, atrophy of the breasts, disappearance of the feminine-type of fat distribution. Masculinisation consists of the development of muscular constitution, increase in the size of clitoris, hirsutism, seborrhoea, acnes, deepening of voice and the turning of sexual interest in the direction of females (BROOKSBANK, 1961). In simplified form: virilism = hirsutism + amenorrhoea (JULESZ, 1957). However, even this simple differentiation cannot be applied to every case as in the so-called "simple hirsutism" there are often oligo- or amenorrhoea and acnes, *i. e.* changes indicative of endocrine disturbances.

Hirsutism accompanies many endocrinological entities and certain non-endocrine conditions as well. In some cases genetical factors also play a role (SHERESHEVSKY et al., 1949; BISSEL, 1951; GREENBLATT, 1953). The common

cause is considered to be an overproduction of androgenic steroids, either absolute or in relation to oestrogen production. However, excessive amounts of androgens may be circulating in males with sparse beard growth (HAMILTON, 1958).

The androgenic steroids and their metabolites are estimated mostly in urine and less often in blood, forgetting that the site of hirsutism is the skin, or more exactly the hair follicles. There is ample evidence to prove that the skin is rich in cholesterol and some data suggest that it has a role in steroid metabolism (MOORE et al., 1952; IDLER et al., 1952; MILLER et al., 1954, HOLLÓ et al., 1959).

The simplest test by means of which attempts have been made to classify the types of hirsutism is the determination of the urinary neutral 17-ketosteroids. Although there seems to be some relationship between 17-ketosteroid excretion and the measure of hirsutism, this is not the rule (FERRIMAN et al., 1957; BROOKSBANK, 1961), as in simple hirsutism the total amount of neutral 17-ketosteroids is not increased significantly, or may even be abnormally low (BISSEL et al., 1945).

More information was expected from studies concerned with the fractions of 17-ketosteroids. SALTER et al. (1946) were among the first to study the urinary 3 alpha and 3 beta-hydroxy-17-ketosteroid fractions, and found them to be normal in cases of simple hirsutism, while later SACCHI et al. (1954), GALLAGHER et al. (1958), as well as HERRMANN et al. (1960), however, found a significant increase in certain ketosteroid fractions. Accordingly, in most of the cases of simple hirsutism of unknown aetiology there occur such disturbances of androgenic steroid metabolism, as are found in the cases of adrenocortical hyperplasia or tumours.

Certain diseases of the ovaries (microcystic degeneration, virilising tumours) are accompanied by hirsutism or virilism. The main problem of decisive significance in prescribing treatment is to differentiate adrenal virilism from ovarian virilism. If hirsutism cannot be traced back to adrenocortical or ovarian disturbances, the condition is usually termed idiopathic.

Estimation of the 17-ketosteroid, glycocorticoid, oestrogenic and gestagenic fractions associated with stimulative and inhibitory interventions make it often possible to differentiate adrenocortical virilism from ovarian virilism, and represent a great step forward in our knowledge of adrenocortical and other steroid metabolism. From the vast literature let us only refer to the investigations of PERLOFF et al. (1957), MASUDA (1957), GALLAGHER et al. (1958), NABARRO et al. (1958), PERLOFF et al. (1958), JAYLE et al. (1959), HERRMANN et al. (1960), CHARVAT et al. (1961), CSILLAG et al. (1962) and CHARVAT et al. (1962).

However important had been the data supplied by the analysis of urinary steroids, it had not disclosed a single fundamental causative factor of hirsutism,

nor ruled out the role of some unknown androgens. If we ignore endocrine hierarchy, we shall never be able to understand the essence of hirsutism and virilism. In that hierarchy, the central nervous system reigns. It suffices to mention the cases of hirsutism released by encephalitis, or the case of BUSH et al. (1959), in which a love affair had elicited the condition. The next centre is the adenohypophysis, which influences through ACTH and the different gonadotropins the various zones of the adrenal cortex and the cells of the ovary producing different steroids, including androgens. The steroids of these reacting glands act on the peripheral tissues and cells. According to our working hypothesis, the ultimate, decisive site of the effect is in the enzyme systems of these cells, in the enzyme systems of the hair follicles in the case of hirsutism. The realization of the androgenic effect depends on the reactivity of these cells to different stimuli. On the basis of theoretical considerations hirsutism will develop in a woman at the "cellular level", when too much androgen enters the hair follicles from the blood, or when the cells of the hair follicles combine more firmly with, or are more sensitive to, androgens, or break down oestrogens at a faster rate, than under normal conditions. Studies of these considerations will certainly bring us nearer to an understanding of the so-called idiopathic hirsutisms and make it possible to elucidate the Seabright-bantam syndrome-like forms. In order to be able to carry out such investigations and to evaluate them, we had to collect evidence in our cases of hirsutism. These will be described below.

Experimental

Methods

The material to be discussed consisted of 49 patients with hirsutism. They were divided into the following groups.

(i) Adrenocortical origin
  a) of the Cushing type (9 patients),
  b) of the adrenogenital type (14 patients).
(ii) Ovarian origin (10 patients).
(iii) Idiopathic (16 patients).

The classification was based on the following.

1. Data mentioned in the history, with special reference to hair growth and other skin manifestations (seborrhoea, acne), menstruation, sterility, libido.

2. Physical examination, with attention devoted to the amount and masculine distribution of hair, or masculine-type baldness; fresh, wide or many purple striae, girdle obesity, moon-face, and a shift of constitution toward the masculine; blood pressure, body weight as related to height.

3. Of the results of laboratory tests exclusively the dextrose tolerance curves were taken into consideration.

4. Adrenal radiographs made after presacral air insufflation (in almost every case) and radiological signs of osteoporosis.

5. Gynaecological opinion relative to the size of the clitoris and ovaries. In patients with menstrual disorders vaginal cytology was studied in the premenstrual period, to determine the character of the disturbance.

Relying upon the above evidence we included into group (i) *a* (Cushing type hirsutism of adrenocortical origin) those patients, whose hirsutism was neither coarse nor extensive, who had girdle obesity, fresh, broad or numerous striae, hypertension, diabetes, or eventually

a protracted diabetoid blood sugar curve. Radiological evidence of osteoporosis and unilateral or bilateral adrenal enlargement confirmed the diagnosis. Patients with Cushing's disease (pituitary basophilism) or Cushing's syndrome were equally represented in this group.

In group (i)*b* (hirsutism of adrenocortical origin and of the adrenogenital type) belonged the patients who were not obese but of muscular constitution, with coarse, masculine hirsutism, with or without masculine baldness, oily skin with acnes but no striae, a deep voice, hypertrophic clitoris, no osteoporosis. In some cases one of the adrenals was enlarged. Most, but not necessarily all, of the above criteria were present in every patient classified in this group.

(ii) To the ovarian group were classified those patients with hirsutism, in whom gynaecological examination detected enlargement of one ovary or both ovaries. The vaginal cytology, if cyclic, deviated from the normal pattern, for example it showed proliferation during the premenstrual period. Most patients suffered from secondary amenorrhoea, none of them had children and in every one of them there was microcystic degeneration or a cyst of the ovary.

(iii) Cases lacking all of the above criteria were classified as idiopathic.

The classification was based exclusively on the enumerated criteria, not taking into consideration the urinary steroids.

The urine was tested for steroids in the following way.

Neutral total 17-ketosteroids (17-ks) were determined on three consecutive days, by the method of HOLTORFF and KOCH (1940), as modified by FAREDIN et al. (1956), and the mean of the results was calculated.

Urinary 17-ks was fractionated by chromatography on aluminium hydroxide according to DINGEMANSE et al. (1946, 1952), by the method of FAREDIN et al. (1957, 1958), combining the procedure of ROBINSON and GOULDEN (1949) and the solvent system of POND (1951). From the chromatogram dissolved off the aluminium hydroxide the following 17-ks fractions can be separated from peaks I to VIII distinguished according to DINGEMANSE:

The beta-17-ks fraction (beta-17-ks-fr.), which contains principally isoandrosterone, dehydroisoandrosterone, isoandrostene-6 (beta)-ol-17-on, artifacts of these steroids, mainly 3-chlorodehydroisoandrosterone and delta^{3,5}-androstanedione-17-on (peaks I + II + III).

The androsterone fraction (A-fr) is composed mainly of androsterone and eventually of delta^{9}-androstene-3 (alpha)-ol-17-on, arising from 11-hydroxyandrosterone on boiling with HCl (peak IV).

The etiocholanolone fraction (E-fr.) is composed mainly of etiocholanolone and an artefact, delta^{9}-etiocholene-3 (alpha)-ol-17-on, formed from 11-hydroxyetiocholanolone. The fractions of androsterone and etiocholanolone together form the alpha$_1$ 17-ks fraction.

The 11-oxygenated-17-ketosteroid fraction (11-oxyg.-17-ks fr.) contains 11-hydroxyandrosterone, 11-hydroxyetiocholanolone, as well as 11-ketoandrosterone and 11-ketoetiocholanolone (peaks VI and VII). This fraction corresponds to the one called in the literature alpha$_2$-17-ks fraction.

In the 17-ks-fr. assays we used that one of the three urine samples, which showed the 17-ks values nearest to the mean. The results for the single 17-ks fractions are given in terms of androsterone mg/24 hrs.

The total 17-hydroxycorticosteroids (17-OH-CS) excreted in urine (Porter-Silber chromogens) were determined on the basis of the Porter-Silber colour reaction (1950), by the method of GLENN and NELSON (1953), as simplified by FAREDIN et al. (1962). Employing ALLEN's correction (1950), we determined the urinary cortisone, cortisol, 11-desoxycortisol, 6-beta-hydroxycortisol, as well as the di- and tetrahydrated derivatives of these steroids together. The results are expressed as cortisol mg/24 hrs., calculating the mean of the values obtained on three consecutive days.

Pregnanediol excretion was studied on the basis of the method described by SOMMERVILLE et al. (1948), on three consecutive days. The results obtained were averaged, and pregnanediol excretion was judged (normal, pathologically increased or decreased) with regard to the menstruation cycle.

Of the oestrogen fractions, oestrone, oestradiol-17-beta and oestriol excretion was studied by the method of BROWN (1955), as modified by BROWN et al. (1957). We determined the mean of the results obtained on two consecutive days and judged the measure of oestrogen excretion (normal, low) with regard to the menstrual cycle.

The steroid assays described were made in 24-hour urine samples collected without preservative and stored in a cool place.

Results

Total 17-ketosteroid

The results are presented in *Table I.* If we analyze the total 17-ks values according to the number of patients in the single groups showing excretion rates exceeding the normal, it will be seen that in the Cushing-type group this occurred in 3 of 9 cases, in the adrenogenital group in 11 of 14 cases, in the ovarian group in 2 of 10 cases, and in the idiopathic group in 2 of the 16 patients.

Table I

Urinary excretion of neutral total 17-ketosteroids in hirsutism

	Age, years	Number of patients tested	Number of patients with increased excretion*	17-ks mg/24 hrs	
				range	mean mg/24 hrs
(i)*a* Cushing-type (adrenocortical origin)	18—36	9	3	8.7 to 27.7	14.8
(i)*b* Adrenogenital-type (adrenocortical origin)	8—47	14	11	9.3 to 27.7	19.3
(ii) Ovarian aetiology	18—35	10	2	8.2 to 20.8	14.0
(iii) Idiopathic hirsutism.....	18—41	16	2	8.4 to 15.6	11.7
(iv) Normal females.....	17—50	33	—	5.0 to 15.0	8.9

* 17-ks excretion exceeding 15.0 mg in 24 hours.

Comparison of the results with those obtained in a group of 33 normal females revealed that in hirsutism mean 17-ks excretion was invariably high, most markedly so in hirsutism of the adrenogenital type.

17-*ketosteroid fractions*

The results of the beta-17-ketosteroid fraction assays are shown in *Table II.* Pathologically increased excretion of this fraction could be demonstrated in one of the 6 patients with Cushing-type hirsutism and in 8 of the 11 adrenogenital-type cases. Excretion exceeded the upper limit of normal in one patient of the 5 ovarian, and in 5 of the 13 idiopathic, cases.

Comparison with results obtained in normal females showed that beta-17-ks fr. excretion could be considered pathological in the adrenogenital group only.

The data for the androsterone fraction are presented in *Table III.* Increased excretion occurred in 2 of the 6 Cushing-type patients, in 6 of the 11 with adrenogenital hirsutism, in 2 of the 5 ovarian cases and in 2 of the 13 idiopathic ones.

Table II

Urinary excretion of beta-17-ketosteroid in hirsutism

	Age, years	Number of patients tested	Cases with pathological excretion*	24-hour excretion, mg, range	Mean mg/24 hrs.
(i)*a* Cushing-type (adrenocortical aetiology)	18—36	6	1	1.96 to 5.35	3.74
(i)*b* Adrenogenital-type (adrenocortical aetiology)	8—47	11	8	1.93 to 9.86	6.82
(ii) Ovarian etiology	27—35	5	1	3.03 to 5.10	4.20
(iii) Idiopathic hirsutism	18—41	13	5	2.40 to 5.86	4.49
(iv) Normal females	18—48	10	—	1.80 to 4.90	3.44

* Number of patients excreting more than 4.90 mg of beta-17-ks in 24 hours.

Table III

Urinary excretion of androsterone in hirsutism

	Age, years	Number of patients tested	Pathological excretion*	24-hour excretion, mg, range	Mean mg/24 hrs.
(i)*a* Cushing-type (adrenocortical aetiology)	18—36	6	2	1.32 to 7.02	3.53
(i)*b* Adrenogenital-type (adrenocortical aetiology)	8—47	11	6	1.93 to 9.46	4.43
(ii) Ovarian aetiology	27—35	5	2	2.98 to 5.42	3.99
(iii) Idiopathic hirsutism	18—41	13	2	1.42 to 5.29	2.75
(iv) Normal females	18—48	10	—	1.30 to 4.00	2.43

* Number of patients excreting more than 4.00 mg of androsterone in 24 hours.

The mean value differed significantly from the one obtained in the group of normal women exclusively in the adrenogenital group, although excretion was higher than normal in every group of hirsutism.

The results of the etiocholanolone assays are shown in *Table IV*. Increased excretion was found in 5 of the 6 Cushing-type, 10 of the 11 adrenogenital and in 4 of the 5 ovarian patients. In the idiopathic group the difference was less marked, because only 5 of the 13 patients showed an increased output.

Comparison with normal values revealed a statistically significant increase of etiocholanolone excretion in every hirsutism group.

The results of the 11-oxygenated-17-ketosteroid assays are shown in *Table V*. Increased urinary excretion was demonstrated in 3 of the 6 Cushing patients, in 5 of the 11 adrenogenital, 1 of the 5 ovarian and 2 of the 13 idiopathic cases.

Table IV

Urinary excretion of etiocholanolone in hirsutism

	Age, years	Number of patients tested	Pathological excretion*	24-hour exretion, mg, range	Mean mg/24 hrs.
(i)*a* Cushing-type (adrenocortical aetiology)	18—36	6	5	2.75 to 7.20	5.51
(i)*b* Adrenogenital-type (adrenocortical aetiology)	8—47	11	10	2.99 to 8.10	6.07
(ii) Ovarian aetiology	27—35	5	4	2.99 to 5.42	4.60
(iii) Idiopathic hirsutism	18—41	13	5	2.94 to 6.55	4.40
(iv) Normal females	18—48	10	—	1.50 to 4.30	2.98

* Number of patients excreting more than 4.30 mg of etiocholanolone in 24 hours.

Table V

Urinary excretion of the 11-oxygenated-17-ketosteroid fraction in hirsutism

	Age, years	Number of patients tested	Pathological excretion*	24-hour excretion, mg, range	Mean mg/24 hrs.
(i)*a* Cushing-type (adrenocortical aetiology)	18—36	6	3	1.35 to 3.93	2.46
(i)*b* Adrenogenital-type (adrenocortical aetiology)	8—47	11	5	0.81 to 4.63	2.18
(ii) Ovarian aetiology	27—35	5	1	1.00 to 2.35	1.51
(iii) Idiopathic patients	18—41	13	2	0.54 to 2.39	1.24
(iv) Normal females	18—48	10	—	0.40 to 1.80	0.96

* Number of patients excreting more than 1.80 mg of the 11-oxygenated-17-ketosteroid fraction in 24 hours.

Excretion was highest in the Cushing-type and adrenogenital-type groups. It was increased in the ovarian and idiopathic groups, too, with the smallest difference in the idiopathic group.

Total 17-*hydroxycorticosteroid*

The results obtained for 17-hydroxycorticosteroids, or 17,21-dihydroxy-20-ketosteroids, are to be found in *Table VI*. Increased was the excretion in 6 of the 8 Cushing-type patients, 5 of the 12 adrenogenital, 1 of the 7 ovarian and 2 of the 14 idiopathic cases.

Comparison with normal subjects showed the highest increase in the adrenocortical group; the highest values were found in the Cushing-type group.

Table VI

Urinary total 17-hydroxycorticosteroid excretion in hirsutism

	Age, years	Number of patients tested	Pathological excretion*	24-hour excretion, mg, range	Mean mg/24 hrs.
(i)*a* Cushing-type (adrenocortical aetiology)	18—36	8	6	2.98 to 18.98	9.90
(i)*b* Adrenogenital-type (adrenocortical aetiology)	8—40	12	5	2.51 to 10.01	5.53
(ii) Ovarian aetiology	18—33	7	1	2.99 to 7.93	4.35
(iii) Idiopathic hirsutism	18—41	14	2	1.25 to 7.68	4.08
(iv) Normal females	18—50	20	—	1.50 to 6.00	3.90

* Number of patients excreting more than 6.0 mg of total 17-OH-corticosteroids in 24 hours.

Pregnanediol

The results are shown in *Table VII*. Taking into consideration the period of menstruation, pathologically increased, normal and pathologically decreased excretion rates were distinguished. Thus, excretion was increased in 2, and normal in 5, of the Cushing-type 7 patients. Of the 12 adrenogenital patients 6 showed increased, 4 normal and 2 low excretion. In the ovarian group one patient showed a pathological increase and six a pathological decrease. Among the 14 idiopathic cases normal excretion was found in 11 and a pathologically decreased value in 3 cases; these results were closest to those obtained in normal subjects.

As the results indicate, pregnanediol excretion was nearest to normal in the idiopathic group, and farthest from it in the ovarian group. In most cases pregnanediol excretion was pathologically low. The distribution was most variable in the adrenogenital group, in which 50 per cent of the patients showed increased excretion.

Table VII

Urinary pregnanediol excretion in hirsutism

	Age, years	Number of patients tested	Number of patients showing high, low, normal pregnanediol excretion			Range mg/24 hrs.
(i)*a* Cushing-type (adrenocortical aetiology) ..	18—36	7	—	2	5	2.33—6.09
(i)*b* Adrenogenital-type (adrenocortical aetiology)	8—47	12	6	2	4	1.33—74.3
(ii) Ovarian aetiology ...	18—33	7	1	6	—	1.12—7.87
(iii) Idiopathic hirsutism .	18—41	14	—	3	11	1.10—5.55
(iv) Normal females	18—45	20	—	—	20	1.40—6.00

Oestrogen fractions

These are shown in *Table VIII*. Oestrogens were assayed in 24-hour urine samples collected on two consecutive days. The mean values for oestrone, oestradiol-17-beta and oestriol were designated low or normal, with regard to the menstrual cycle. The results are presented in *Table VIII*.

Oestrone excretion was low in 3 of the 4 Cushing-type patients. Of the 7 adrenogenital patients 4 showed low, and 3 normal, excretion. The one patient

Table VIII

Excretion of oestrogen fractions in hirsutism

	Number of cases	Name	Age, years	Oestrone excretion µg/24 hrs	Evaluation	Oestradiol-17 beta excretion µg/24 hrs	Evaluation	Oestriol excretion µg/24 hrs	Evaluation
(i)*a* Cushing-type (adrenocortical aetiology)	1**	A. I.	35	0.6	low	1.7	low	37.8	normal
	2*	B. Gy.	36	3.1	normal	1.7	normal	8.4	normal
	3**	P. M.	30	2.0	low	2.2	normal	23.6	normal
	4**	F. E.	18	1.6	low	2.7	normal	10.6	normal
			Mean:	1.8	Mean:	2.1	Mean:	20.1	
(i)*b* Adrenogenital-type (adrenocortical aetiology)	1**	K. Zs.	21	0.4	low	0.0	low	20.5	normal
	2*	M. A.	15	0.7	low	0.7	low	2.0	low
	3**	P. V.	23	2.0	low	1.6	low	20.9	normal
	4*	B. É.	20	1.9	normal	2.4	normal	7.4	normal
	5**	K. M.	20	2.8	normal	0.0	low	7.8	normal
	6**	P. E.	22	2.0	low	1.9	normal	7.6	normal
	7**	P. A.	31	3.0	normal	3.7	normal	28.5	normal
			Mean:	1.8	Mean:	1.5	Mean:	13.5	
(ii) Ovarian group	1*	A. Gy.	29	1.9	low	1.7	low	15.5	normal
(iii) Idiopathic group	1*	B. M.	23	2.8	normal	1.8	normal	3.0	low
	2*	Cs. E.	18	0.6	low	0.0	low	3.5	low
	3**	Cs. I.	18	2.0	low	1.8	normal	14.9	normal
	4*	H. M.	24	1.2	low	0.5	low	24.9	normal
	5*	T. Gy.	41	5.3	normal	2.9	normal	27.7	normal
	6**	B. E.	40	1.9	low	1.6	low	19.8	normal
	7*	R. M.	21	2.8	normal	1.9	normal	18.8	normal
			Mean:	2.4	Mean:	1.5	Mean:	16.1	

* Tests made during first half of menstrual cycle.
** Tests made during second half of menstrual cycle.

with hirsutism of ovarian origin showed low excretion. Of the 7 patients in the idiopathic group excretion was normal in 3 and low in 4.

Excretion of oestradiol-17-beta fraction was as follows. Cushing-type group, 3 normal, 1 pathologically low, adrenogenital group, 4 pathologically low, 3 normal; one patient with ovarian hirsutism, low, idiopathic hirsutism group, 4 normal and 3 pathologically low.

Excretion of oestriol was normal in all four Cushing-type patients, in 6 of the 7 adrenogenital patients, in the one ovarian patient and in 5 of the 7 patients in the idiopathic group.

A comparison of the oestrogen excretion values with those obtained in normal females of about the same age *(Table IX)* revealed that in hirsutism less oestrone is excreted, with oestradiol-17-beta around the lower limit of normal and a normal excretion of oestriol.

Table IX

Urinary excretion of oestrogen fractions in normal females

Normal females	Number of cases	Oestrogen excretion μg/24 hrs		Oestradiol-17-beta excretion, μg/24 hrs		Oestriol excretion μg/24 hrs	
		range	mean	range	mean	range	mean
During first half of menstrual cycle	8	1.80—6.60	3.37	1.20— 9.30	3.18	4.20—25.20	10.79
During second half of menstrual cycle	8	2.10—6.30	3.60	1.80—13.20	3.47	4.50—44.02	20.03

Discussion

Our aim in studying urinary steroids was to determine whether a characteristic excretion of some steroid could be demonstrated in the groups of hirsutism set up on the basis of the clinical picture and other diagnostic examinations. In other words, whether steroid assays would, and how reliably, facilitate diagnostic distinction.

The excretion of neutral 17-ketosteroids was studied in detail, because they incorporate many steroids of androgenic action. The results showed higher excretion values in every group of hirsutism than in the group of normal women. Urinary 17-ketosteroid excretion showed the greatest deviation in the adrenogenital group, yet pathological increases of 17-ketosteroid excretion were found, though less often in the other groups as well. Normal 17-ketosteroid values, too, occurred in every group, in the largest number in the idiopathic group.

Similar results have been obtained for the urinary excretion of beta-17-ketosteroid and androsterone fractions. The mean values were higher in every group of hirsutism than in the normal group; the highest excretion rates oc-

curred in the adrenogenital group. Etiocholanolone excretion was not characteristic of any of the groups; increased amounts were excreted in every group. The values for 11-oxygenated 17-ketosteroid diverged from the normal mean particularly in hirsutism of adrenocortical origin, although the ovarian and idiopathic forms, too, showed increased excretion rates. Normal values for this fraction, however, occurred in every group, most often in the ovarian and idiopathic groups.

The results for 17-hydroxycorticosteroid, which supply information mainly on the excretion of hydrocortisone, cortisone and their di- and tetrahydrated derivatives, diverged most markedly from the normal mean in the group of patients with hirsutism of adrenocortical origin, especially in the Cushing-type form. Except for one or two cases, excretion was normal in the ovarian and idiopathic groups. These results merit attention, because in possession of the evidence supplied by them and the result for total 17-ketosteroid excretion it is possible to conclude to the adrenocortical origin of a given case of hirsutism.

Pregnanediol was assayed by the less specific Sommerville method. As it has been shown by LORAINE (1958) and BROOKSBANK (1961), this method determines in the urine pregnanediol together with pregnanetriol. In the adrenogenital syndrome the urinary excretion of pregnanetriol is pathologically increased (FINKELSTEIN, 1959; HILL, 1960; COX, 1960) and therefore the high pregnanediol excretion found in most of our adrenogenital-type patients may be ascribed to a disturbing action of pregnanetriol. Otherwise, in this group both normal and pathologically low excretion values were obtained. The same applies to the other groups of hirsutism, and therefore the assay in question does not allow their differentiation.

In the pathogenesis of hirsutism many authors attribute significance to the excretion of oestrogens (GILBERT-DREYFUS et al., 1951; AUDIT, 1952 a and b). The cases of hirsutism with normal or near-normal androgen excretion are brought into correlation with a diminished excretion of oestrogens (relative hyperandrogenism). The studies concerning oestrogen excretion in hirsutism yielded little evidence, owing to the lack of a suitably specific method. We used the method of BROWN (1955) modified by BROWN et al., (1957), this being the one generally accepted in the literature. Although our data do not allow final conclusions, they nevertheless showed that in hirsutism the urinary excretion of oestrone and oestradiol-17 beta is lower than normal. Oestriol excretion was, however, normal in every group of hirsutism. Although they may be useful in studies concerned with the pathogenesis of hirsutism, our data concerning oestrogen excretion did not make it possible to differentiate between the different types of hirsutism.

Thus, tests of urinary steroid excretion are a help in the separation of hirsutism due to adrenocortical disturbances from those due to other changes,

but make no reliable distinction possible. More can be expected from steroid assays coupled with stimulation and inhibition, as indicated by the recent investigations of CSILLAG et al. (1962) and CHARVAT et al. (1962).

Although our results have revealed no new possibility of an improved differential diagnosis, they allow some insight into the disturbances of steroid metabolism accompanying hirsutism. The behaviour of the etiocholanolone, oestrone and oestradiol-17 beta fractions merits particular attention. In every group of hirsutism the urinary excretion of etiocholanolone was pathologically increased, and that of oestrone and oestradiol-17 beta decreased. Whence this excessive etiocholanolone production in hirsutism? According to present knowledge, the precursors of etiocholanolone, dehydroisoandrosterone, delta^4-androstendione and testosterone may be produced by the adrenal cortex and the ovary (MASON and KEPLER, 1945; MAHESH and GREENBLATT, 1961, 1962; GOLDZIEHER and AXELROD, 1962). These authors have shown that, in certain diseases of the ovary, steroid deviates biosynthesis from the normal and takes place *via* 17 alpha-hydroxy-delta^5-pregnenolone, from dehydroisoandrosterone to delta^4-androstendione. These results have been confirmed by AXELROD and GOLDZIEHER (1962) who incubated ovarian tissue made with progesterone-4-C^{14}, delta^5-pregnenolone-4-C^{14} and testosterone-4-C^{14} and found the activity of the ovarian aromatizing, 17-hydroxylating and 3 beta-ol-dehydrogenase enzymes to diminish significantly. This deficient enzymic activity is responsible for the decreased excretion of oestrogen and the increased excretion of dehydro-isoandrosterone, androsterone, etiocholanolone and testosterone. The results of DORFMAN (1963) are in harmony with these data. According to DORFMAN, in females suffering from hyperandrogenism, and even in idiopathic hirsutism, the blood testosterone level is increased even when urinary total 17-ketosteroid excretion is normal. The increased production of testosterone would be responsible for the principal clinical manifestations of hyperandrogenism. Although we made no testosterone studies, in view of the fact that according to CALLOW (1939) and BIRKE and PLANTIN (1954) androsterone and etiocholanolone are the chief metabolites of testosterone, our results tend to support those obtained in the above investigations. On the basis of our results and in the light of data reported in the literature we surmise that, although not specifically, the increased excretion of etiocholanolone belongs to the essence of hirsutism.

On the basis of the afore-mentioned investigations of AXELROD and GOLDZIEHER (1962), we ascribe the low urinary oestrone and oestradiol-17-beta excretion to a diminished activity of the ovarian aromatizing enzymes.

The extensive studies of steroid metabolism in patients with hirsutism have revealed various disturbances, but their pathogenetic significance is not clear. Some unknown androgen or an altered reactivity of peripheral tissues or cells may perhaps also have a role. Studies of the problem are rendered diffi-

cult by the fact that very little is known about the biochemistry, physiology and pathology of the hair follicle. Not even that is known whether hair growth is influenced by the androgenic steroids. Investigations into this problem may yield interesting data concerning the development of hirsutism. Such investigations are in progress.

REFERENCES

1. Allen, W. M.: J. clin. Endocr. **10,** 71 (1950).
2. Audit, J. F.: Gynéc. et Obstét. **51,** 236 (1952a).
3. Audit, J. F.: Presse méd. **60,** 554 (1952b).
4. Axelrod, L. R., Goldzieher, J. W.: J. clin. Endocr. **22,** 431 (1962).
5. Birke, G., Plantin, L. O.: Acta med. scand. Suppl. **148,** 291 (1954).
6. Bishop, P. M. F.: Recent Advances in Endocrinology. Churchill, London 1954.
7. Bissel, G. W., Williams, R. H.: Ann. intern. Med. **22,** 773 (1945).
8. Bissel, G. W.: Ann. N. Y. Acad. Sci. **53,** 742 (1951).
9. Brooksbank, B. W. L.: Physiol. Rev. **41,** 623 (1961).
10. Brown, J. B.: Biochem. J. **60,** 185 (1955).
11. Brown, J. B., Bulbrook, R. D., Greenwood, F. C.: J. Endocr. **16,** 49 (1957).
12. Bush, I. E., Mahesh, V. B.: J. Endocr. **18,** 1 (1959).
13. Callow, N. H.: Biochem. J. **33,** 931 (1939).
14. Charvát, J., Gregorová, I., Kandráč, M.: Endokrinologie **43,** 117 (1962).
15. Charvát, J., Gregorová, I., Kandráč, M.: Čas. Lék. čes. **100,** 956 (1961).
16. Cox, R. I.: Acta endocr. (Kbh.) **33,** 477 (1960).
17. Csillag, M., Avar, Z., Vatai, M.: Orv. Hetil. **103,** 1302 (1962).
18. Dingemanse, E., Huis in't Veld, L. G., de Laat, B. M.: J. clin. Endocr. **6,** 355 (1946).
19. Dingemanse, E., Huis in't Veld, L. G., Hartog-Katz, S. L.: J. clin. Endocr. **12,** 66 (1952).

19/a. Dorfman, R. I.: Obstetric. a. Gynec. Surv. **18,** 65 (1963).

20. Dorfman, R. I., Shipley, E. A.: Androgens. Wiley, New York 1956.
21. Faredin, I., Novaszel, F., Kende, E.: Kísérl. Orvostud. **8,** 438 (1956).
22. Faredin, I., Novaszel, F., Blahó, Gy., Kende, E.: Kísérl. Orvostud. **9,** 225 (1957).
23. Faredin, I., Blahó, Gy.: Orv. Hetil. **49,** 1780 (1958).
24. Faredin, I., Szabadai, I., Simor, I.: Kísérl. Orvostud. **14,** 549 (1962).
25. Ferriman, D., Thomas, P. K., Purdie, A. W.: Brit. med. J. **2,** 1410 (1957).
26. Finkelstein, M.: Acta endocr. (Kbh.) **30,** 489 (1959).
27. Gallagher, T. F., Kappas, A., Hellman, L., Lipsett, M. B., Pearson, O. H., West, C. D.: J. clin. Invest. **37,** 794 (1958).
28. Gilbert-Dreyfus, Zara, M., Alexandre, C.: Sem. Hôp. Paris **27,** 1398 (1951).
29. Glenn, E. M., Nelson, D. H.: J. clin. Endocr. **13,** 911 (1953).
30. Goldzieher, J. W., Axelrod, L. R.: J. clin. Endocr. **22,** 435 (1962).
31. Greenblatt, R. G.: Amer. J. Obstet. Gynec. **66,** 700 (1953).
32. Hamilton, J. B. I.: The Biology of Hair Growth. Ed. W. Montagna and R. A. Ellis, Academic Press, New York 1958, p. 399.
33. Herrmann, W., Buckner, F., Morris, J.: Fertil. and Steril. **11,** 74 (1960).
34. Hill, E. E.: Acta endocr. (Kbh.) **33,** 230 (1960).
35. Holló, I., Julesz, M., Koref, O., Megyesi, K.: Acta med. Acad. Sci. hung. **13,** 147 (1959).
36. Holtorff, A. F., Koch, F. C.: J. biol. Chem. **135,** 377 (1940).
37. Idler, D. R., Baumann, C. A.: J. biol. Chem. **195,** 623 (1952).
38. Jayle, M. F., Malassis, D., Pinaud, H.: Acta endocr. (Kbh.) **31,** 1 (1959).
39. Julesz, M.: A neuroendokrin betegségek kórtana és diagnosztikája. Akadémiai Kiadó, Budapest 1957.
40. Loraine, J. A.: The Clinical Application of Hormone Assay. E. a. S. Livingstone Ltd. Edinburgh 1958.
41. Mahesh, V. B., Greenblatt, R. B.: Nature (Lond.) **191,** 888 (1961).
42. Mahesh, V. B., Greenblatt, R. B.: J. clin. Endocr. **22,** 441 (1962).
43. Mason, H. L., Kepler, E. J.: J. biol. Chem. **160,** 255 (1945).
44. Masuda, M.: J. clin. Endocr. **17,** 1181 (1957).
45. Miller, W. L., Jr., Baumann, C. A.: Proc. Soc. Exp. Biol. (N. Y.) **85,** 561 (1954).
46. Moore, P. R., Baumann, C. A.: J. biol. Chem. **195,** 615 (1952).

47. Nabarro, J. D. N., Moxham, A., Slater, J. D. H., Walker, G.: Proc. roy. Soc. Med. **51**, 552 (1958).
48. Perloff, W. H., Hadd, H. E., Channick, B. J., Nodine, J. H.: Arch. intern. Med. **100**, 981 (1957).
49. Perloff, W. H., Channick, B. J., Suplick, B., Carrington, E. R.: J. A. M. A. **167**, 2041 (1958).
50. Pond, M. H.: Lancet **2**, 906 (1951).
51. Porter, C. C., Sibler, R. H.: J. biol. Chem. **185**, 201 (1950).
52. Robinson, A. M., Goulden, F.: Brit. J. Cancer **3**, 62 (1949).
53. Sacchi, F., Natoli, A., Zilli, E.: Rif. med. **68**, 981 (1954).
54. Salter, W. T., Cahen, R. L., Sappington, T. S.: J. clin. Endocr. **6**, 52 (1946).
55. Shereshevsky, N. A., Vasyukova, E. A.: Klinichesk. Med. **26**, 9 (1949).
56. Sommerville, J. T., Marrian, G. F., Kellar, R. J.: Lancet **2**, 89 (1948).

Dr. Miklós Julesz
Dr. Imre Faredin
Dr. István Tóth
Dr. Margit A. Dávid
Dr. Kálmán Kovács

Orvostudományi Egyetem, I. Belklinika, Szeged, Hungary

EINE KLINISCHE METHODE ZUR VERGLEICHUNG DER WIRKUNG VON SPASMOLYTIKA

Von

GY. PETRÁNYI und GY. SZEGEDI

MEDIZINISCHE KLINIK (DIREKTOR: PROF. DR. GY. PETRÁNYI) DER MEDIZINISCHEN UNIVERSITÄT, DEBRECEN

(Eingegangen am 3. April 1963)

1. Die bei durch Arterienkonstriktion bedingten Erkrankungen der unteren Extremität übliche intraarterielle vasodilatorische Behandlung wurde zur Messung der auf die glatte Muskulatur ausgeübten Wirkung von spasmolytischen Mitteln angewendet und die Methode als klinikopharmakologischer Test empfohlen.

2. Mit dem Test wurde eine vergleichende Untersuchung der Wirkung von Papaverin, Ethaverin und eines neuen Derivates, des Dihydroethaverins durchgeführt.

3. Dihydroethaverin übertraf Papaverin und Ethaverin an Wirksamkeit und ermöglichte bei abdominalen Krämpfen eine Einsparung an Betäubungsmitteln.

Der Wirkungsgrad verschiedener Spasmolytika läßt sich nur mit Hilfe pharmakologisch-biologischer Untersuchungen exakt messen. Es ist allgemein bekannt, daß die klinischen Messungen in Fällen von »Magenkrämpfe«, »Gallenkoliken«, »Nierenkoliken« usw. zufolge der außerordentlich großen Mannigfaltigkeit auch bei ein und demselben Individuum unzuverlässig sind. Besonders schwierig gestaltet sich die genaue, objektive Messung zweier pharmakologisch relativ nahestehender Mittel auf Grund von Angaben der Patienten. Die klinische Wertbestimmung, die das eine oder andere Mittel in offenkundig veränderlichen und verschiedenen Zuständen anwendet und als Resultat das Urteil und Angaben von Patienten wertet, kann sich auch im Falle einer großen Anzahl von Untersuchungen nur auf gewisse Meßwerte von beschränkter Bedeutung, wie »gut, mäßig, null« und »prompt, langsam« bzw. »anhaltend, schnell, vergänglich« stützen.

Zwecks Ausschaltung der Subjektivität der Kranken wird die Blind-Methode verwendet; um auch einer Selbsttäuschung des behandelnden Arztes vorzubeugen, bedienen wir uns der als am meisten verläßlich anerkannten Doppelblind-Methode. Diese Methode bewahrt sich aber nur dort, wo wir subjektive Effekte messen und die zu untersuchenden Parameter mit objektiven Meßwerten nicht zu ermitteln sind. Die für Untersuchungen der Spasmolytika angewandte Doppelblind-Methode weist auch etliche solche Voraussetzungen auf, die kaum zu erfüllen sind und mangels derer irrtümliche Resultate entstehen können.

Wir waren bemüht, ein klinisches Meßverfahren auszuarbeiten, das den spasmolytischen Effekt mittels Meßzahlen charakterisiert. Unserer Ansicht nach ist für diesen Zweck die nach intraarterieller Injektion zustandekommende Gefäßerweiterung am besten geeignet. Auf diese Art können die Erwärmung der Haut, Grad und Ausdehnung der Hyperämie, eventuell die Menge des durchströmenden Blutes mittels Plethysmographie und Oszillometrie bzw. Oszillo-

graphie gemessen werden. Zur Prüfung der Methode wurde in den gegenwärtigen Untersuchungen die Wirkung eines neuen Spasmolytikums, das Dihydroethaverin, mit der von Ethaverin bzw. Papaverin verglichen.

Methodik

Die intraarterielle Verabfolgung peripher wirkender spasmolytischen Mittel ist ein klinisches Routineverfahren (KUSCHINSKY) bei Obstruktionserkrankungen der Arterien der unteren Extremität (Atherosclerosis obliterans, Endarteritis obliterans). Die Injektion wird mit einer dünnen Nadel in der Inguinalbeuge unter dem Poupartschen Band, in die Art. femoralis gegeben.

Es handelte sich um Patienten, bei welchen die Anwendung intraarterieller Vasodilatatoren wegen der fortgeschrittenen arteriellen Obstruktion begründet war; der Oszillationswert war stark herabgesetzt, an den Zehen traten trophische Störungen in Erscheinung, und neben einer ausgesprochenen Claudicatio intermittens traten bereits im Ruhezustand Parasthesien auf. In einem solchen Zustand führt die Förderung der Zirkulation zur Besserung. Die kollateralen Arteriolen sind jedoch in der Zone der Gefäßobliteration meistens spastisch. Von der Stelle der Gefäßverengerung abwärts ist der Fuß kühler und blasser. Die Wirkung eines auf die kleinen Arterien erweiternd wirkenden Mittel ist durch den Grad der Hyperämie, durch die Ausdehnung derselben, sowie durch den Anstieg der Hauttemperatur gut meßbar. Zumal die Erkrankung der Arterien beider unteren Extremitäten trotz gewisser Symmetrie nicht vollständig identisch ist, wurden die intraarteriellen Injektionen, im Interesse der genauen Vergleichbarkeit an derselben Seite, jeden zweiten Tag verabreicht.

Wir registrierten mittels eines elektrischen Hautthermometers Umfang und Grad der Hyperämie bzw. deren zeitlichen Verlauf sowie den Verlauf der Erwärmung. Die Oszillometrie wurde systematisch durchgeführt.

Die Messung des Grades der Gefäßerweiterung erfolgte in einem Zimmer von ca 18 °C ständiger Temperatur. Es ist zweckmäßig abzuwarten, bis die Temperatur der unbedeckten Extremität das tiefste ständige Niveau erreicht. Starke Lichtquellen (z. B. beim Photographieren verwendete starke Reflektoren) stören das Messen der Hauttemperatur. Vorangehenden pharmakologischen Untersuchungen gemäß (ISSEKUTZ, DÁVID und GYARMATI, KOVÁCH und MENYHÁRT) soll die spasmolytische Wirkung von Dihydroethaverin die von Ethaverin bzw. Papaverin* in einem Verhältnis 2—3 : 1 übertreffen.

Dihydroethaverin Injektion (»No-Spa« Chinoin Ampulle á 2 ml) enthält 40 mg 6,7,3',4-Tetraaethoxy-1-benzal-1,2,3,4-tetrahydroisochinolinchlorid, eine blaß grünlich-gelbe, nicht hygroskopische, in Wasser gut lösliche Substanz.

Ergebnisse

Die vergleichenden Untersuchungen, die in der beschriebenen Weise an insgesamt 10 Kranken durchgeführt wurden, ergaben folgende Ergebnisse. Bei identischen einmaligen Dosen von 80 mg war die vasodilatorische Wirkung

* Das Dihydroethaverin wurde von der Fabrik chemisch-pharmazeutischer Produkte Chinoin, Budapest, zur Verfügung gestellt.

von Dihydroethaverin am ausdrücklichsten, die von Papaverin und Ethaverin wesentlich schwächer. Die der Wirkung von 80 mg Dihydroethaverin entsprechende Gefäßerweiterung war nur mittels ca 200 mg Papaverin bzw. Ethaverin zu erreichen; somit erwies sich im intraarteriellen Test Dihydroethaverin 2½-mal wirksamer als Ethaverin und Papaverin. Dies entspricht annähernd dem in vorangehenden pharmakologischen Versuchen gewonnenen Wirksamkeitsverhältnis und beweist einerseits, daß Dihydroethaverin auch am Menschen

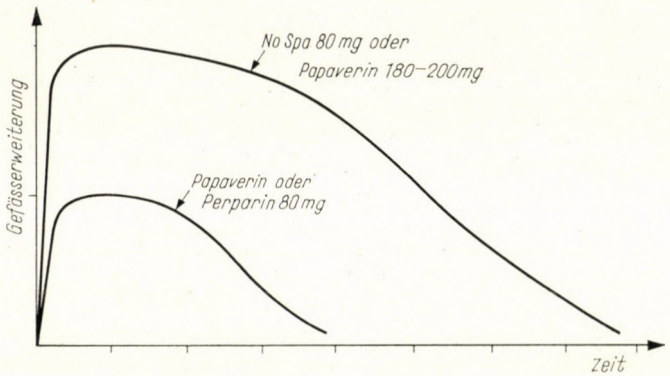


Abb. 1

wirksamer ist als Ethaverin bzw. Papaverin, anderseits begründet es die Feststellung, daß der intraarterielle Test zur Messung der Wirkung, der unmittelbar auf die glatte Muskulatur wirkenden Spasmolytika und Feststellung der relativen Dosisverhältnisse derselben geeignet ist. Die relative Wirksamkeit der geprüften Spasmolytika ist in *Abb. 1* schematisch dargestellt.

Der beschriebene intraarterielle Test besitzt noch einen bedeutenden Vorteil gegenüber den pharmakologischen (Uterushorn, Darmschlinge usw.) Methoden, u. zw. daß auf diesem Wege die objektiven und subjektiven humanen Nebenwirkungen relativ gefahrlos untersucht werden können. Die in die Arterie zugeführte Substanz gelangt vom Unterschenkel mit Blut vermengt in stark verdünntem Zustande in die kritischen Organe (Herz, Lungen, Nervensystem) und ist demzufolge auch zur vorangehenden Untersuchung der Nebenwirkungen der intravenösen Anwendung geeignet.

Nach intraarteriellen Gaben von 80 mg Dihydroethaverin, die eine entsprechende vasodilatorische Wirkung sicherten, waren keinerlei Nebenwirkungen zu beobachten; der Blutdruck zeigte bis zum vollen Verlauf der Gefäßerweiterung und auch danach keine nennenswerte Abweichung. Die intraarterielle Dosis von 80 mg Dihydroethaverin führte bei den Kranken im allgemeinen zu keinen besonderen Unannehmlichkeiten. Demgegenüber beschwerten sich die Kranken ½—2 Minuten nach der intraarteriellen Injektion von 200 mg Papaverin, die zu identischer Gefäßerweiterung führte, oft über Wärme-

gefühl, eigenartiges Allgemeinbefinden, Schwindel, Herzklopfen, wobei gewöhnlich während 1—3 Minuten auch geringe Blutdrucksenkung und Tachypnoe zu beobachten war. Demnach dürfte Dihydroethaverin auch bei intravenöser Anwendung eine günstigere Wirkung entfalten, als Papaverin.

Obzwar die Dosis von 80 mg Dihydroethaverin zum Zwecke der intraarteriellen Gefäßerweiterung im allgemeinen vollauf entspricht, wurde in zwei Fällen bei korpulenten Kranken, bei denen die Gefäßerweiterung des Ausmaßes der Hauterrötung gemäß geringer war, als gewöhnlich, nachfolgend eine Menge von 120 mg angewendet, die ohne Nebenwirkungen zu völliger Rötung der Extremitäten führte.

Bei intraarterieller Verabreichung von Dosen zu 80 mg ergab sich bei Dihydroethaverin nicht nur eine stärkere, sondern auch eine länger anhaltende Wirkung als bei Papaverin, was aus therapeutischem Gesichtspunkte ebenfalls als ein Vorteil zu bewerten sei. Diese anhaltende Wirkung läßt sich naturgemäß auf zwei Ursachen zurückzuführen. Die eine ergibt sich aus dem Umstande, daß eine stärkere Gefäßerweiterung länger anhält; die zweite, daß sich die Wirkung infolge der »besseren Bindung« des Mittels mehr in die Länge zieht. Der intraarterielle Test ist jedoch zur Entscheidung dieser Frage nicht geeignet.

Die ermittelten Wirkungen waren natürlich je nach der Konstitution der Kranken und dem Schweregrad ihres Zustandes ziemlich verschieden; die relativen Wirksamkeitsergebnisse waren jedoch im großen und ganzen identisch.

Nachdem die Dosis bzw. Wirkungsdifferenz an Hand des intraarteriellen Testes verläßlich geklärt war, haben wir uns das Ziel gesetzt, in erster Reihe die Verwendbarkeit des neuen Mittels auf Grund der Nebenwirkungslosigkeit nach intravenöser Verabreichung nachzuweisen. Obzwar intraarteriell sogar höhere Dosen von Dihydroethaverin im Gegensatz zu Papaverin zu keinen Nebenwirkungen führten, wurden bei der intravenösen Anwendung vorerst geringere Dosen erprobt; so wurden Kranken mit Cholelithiase bei milderen spastischen Schmerzen vorerst Dosen von 40 mg gegeben. Da bei 5 Kranken diese relativ geringen Dosen ohne Nebenwirkung zur gänzlichen Behebung der Spasmen und Senkung des Blutdruckes führten, wurden in Fällen von schweren Krampfanfällen Dosen von 80 mg angewandt. Die Geschwindigkeit der intravenösen Einspritzung soll ziemlich langsam geschehen: 2—4 mg/Sek., demnach bei Dosen zu 80 mg: 20—40 Sek. Nur einmal beobachteten wir eine größere aber schnell vorübergehende Blutdrucksenkung mit Schwindelgefühl bei einer hypotonischen Patientin mit Sklerodermie. Man sollte also bei Hypotension auf die intravenöse Zufuhr verzichten bzw. sie sollte nur langsam und mit Vorsicht ausgeführt werden. Die Steinanfälle gehen gewöhnlich mit so heftigen Schmerzen einher, daß die intravenöse Behandlung zum Zwecke prompter Schmerzlinderung begründet ist. Auch bei intravenöser Anwendung war die intensivere spasmolytische Wirkung von Dihydroethaverin, bei identischen Dosen allgemein feststellbar. Die spasmolytische Wirkung trat innerhalb 1—2 Minuten ein, ihre Dauer war jedoch durch die krampfauslösenden Faktoren bedingt.

So z. B. waren 40 mg in einem Falle von cholelithiasischen Krämpfen von guter Wirkung, der Krampf wurde aufgehoben, doch trat er erneut so heftig auf, daß weitere 80 mg verabreicht werden mußten. Die Spasmen lösten sich bald, jedoch nur auf die Dauer von etwa einer halben Stunde. Bei Wiederholung der Dosis wurde der Krampf wieder nur eine halbe Stunde lang gestillt, so daß wiederholt Pethidin und Morphium-Atropin angewendet wurde. Nachdem der Kranke darauf nicht reagierte, wurde zur Operation gegriffen und ein in den Choledochus geklemmter Stein vorgefunden. Natürlich ist auch die energischeste Spasmolyse nur ein symptomatischer Eingriff, der die Krankheit, die die Krämpfe auslöst, nicht heilt. Zu welcher Zeit die Krämpfe nach der Spasmolyse wiederkehren, hängt in erster Reihe von den Faktoren ab, die die Krämpfe auslösen und viel weniger von den spasmolytischen Mitteln. Trotzdem sind die letzteren, als pathogenetische schmerzstillende Mittel zu betrachten, die durch Lösung der Krämpfe wirken und hiebei die Symptome von eventuellen Komplikationen nicht verbergen. Daß Dihydroethaverin nicht nur intramuskulär, subkutan, aber auch intravenös in wirksamen Dosen angewendet werden kann, ist bei mit schweren, abdominalen Krampfanfällen einhergehenden Erkrankungen als ein wesentlicher Vorteil anzuerkennen. Gleichzeitig läßt sich die Wirkung mit intramuskulärer, bzw. subkutaner Verabreichung, oder mit anderen intravenös nicht anwendbaren Spasmolytika kombinieren. Das Mittel wurde intravenös in mehr als 30 Fällen von cholelithiasischen Krämpfen mit guter Wirkung angewendet, wobei

Betäubungsmittel enthaltende Arzneikombinationen vermieden werden konnten. In 8 Fällen von nephrolithiasischen Krämpfen wurde das Mittel ebenfalls erprobt; in einem Falle verschwanden die Krämpfe in wenigen Minuten, ohne Wiederholung derselben; in den anderen Fällen hielt die Schmerzlosigkeit nur 1½—2 Stunden an, wonach sich die Krämpfe wiederholten und weitere Injektionen benötigt wurden (während dessen kamen keine anderen Medikamente zur Anwendung).

Es sei noch der Fall von Frau G. I. angeführt, an deren Finger, durch ziemlich schweres Raynaudsches Syndrom bedingt, trophische Störungen auftraten und sich am letzten Phalanx des II. rechten Fingers ein Gangrän entwickelte; es wurde schon die Amputation geplant, da die Kranke infolge der nur auf Morphin-Atropin reagierenden Schmerzen seit 2—3 Wochen nicht einmal schlafen konnte. Mit Rücksicht darauf, daß die Arterien der oberen Extremität für eine systematische intraarterielle Behandlung nicht geeignet sind, wurde Dihydroethaverin intravenös verabreicht, wodurch sich das Raynaudsche Syndrom wesentlich milderte. Hienach wurde eine systematische Behandlung mit Dihydroethaverin vorgenommen, worauf sich die Schmerzen schnell linderten, das Morphin konnte vermieden werden, Patientin konnte ohne Hypnotika schlafen und das Gangrän begann zu heilen. Im weiteren Verlauf wurde das Mittel intramuskulär, dann subkutan, sodann peroral angewendet; das Gangrän heilte.

LITERATUR

DÁVID, G., GYARMATI, L.: Pharmakologische Dokumentation, Fabrik chemisch-pharmazeutischer Produkte Chinoin, Budapest 1961.

ISSEKUTZ, B.: Pharmakologische Dokumentation, Fabrik chemisch-pharmazeutischer Produkte Chinoin, Budapest 1961.

KOVÁCH, A. G. B., MENYHÁRT, J.: Pharmakologische Dokumentation, Fabrik chemisch-pharmazeutischer Produkte, Budapest 1961.

KUSCHINSKY, G.: s. RATSCHOW.

RATSCHOW, M.: Angiologie, G. Thieme, Stuttgart 1959.

Dr. Gyula PETRÁNYI
Dr. Gyula SZEGEDI
} II. Belklinika, Debrecen, Ungarn.

HISTAMINE CONTENT OF TRANSPLANTABLE MASTOCYTOMA

By

P. GRÓF and G. KELÉNYI

DEPARTMENT OF DERMATOLOGY AND PATHOLOGY,
UNIVERSITY MEDICAL SCHOOL, PÉCS

(Received April 16, 1963)

FURTH's transplantable mouse mastocytoma has been transferred to albino mice of our own breed. The morphological properties of the tumour thus induced have been studied by light and electron microscopy, histological examinations have been made to detect eventual metastasis formation, and the tumours, as well as various organs of the tumorous mice have been assayed by a biological method for histamine content. The following results have been obtained.

1. The FURTH mastocytoma grew in 10 to 15 per cent of the intact adult, and 20 to 40 per cent of previously X-ray-irradiated mice.

2. As determined in 8 cases, the mastocytomas contained from 120.0 μg/g to 1114.0 μg/g histamine. In general, the histamine concentration was lower in well-developed, old tumours, than in young ones weighing less than 1 g. Histamine concentration was higher in the (infiltrated) skin around the tumour than in the skin areas distant from the tumour.

3. The organs of tumorous mice showed no increased histamine concentration. This is interpreted as indicating an absence of metastasis formation, in accordance with the results of the histological studies.

4. Every organ of a LAF_1 mouse tested was very rich in histamine (in agreement with the histological evidence of mast cell infiltration), particularly the liver, which contained 1600 μg/g.

5. The high histamine concentrations found in the liver of the LAF_1 mouse and in the young tumours of the own mice are ascribed to favourable conditions for histamine production in both cases.

The discovery made by RILEY and WEST that most of the histamine in tissues is stored by the tissue mast cells and that the factors liberating histamine cause at the same time the disruption of mast cells, has shown a new direction for histamine research and stimulated the study of the function and biological role of the mast cell. Owing to their appearance and metachromatic staining, the mast cells of EHRLICH (1877) were for decades favoured objects in morphological research, but their physiological significance remained largely unknown. In 1938, MICHELS mentioned about 25 hypotheses put forward in the literature to explain the function of mast cells; none of them was, however, more satisfactory than the one suggested by EHRLICH. The first significant advance in this field was the statement made by JORPES, HOLMGREN and WILANDER (1937) that the metachromatic substance of the mast cell was identical with heparin. Some 15 years later it was discovered that the mast cells store histamine, the significance of which fact was increased by the recognition that the mast cells became degranulated not only in response to the widest variety

of physical or chemical effects, giving off to the environment the substances stored in their cytoplasm or in the granules, but the same happens with the sensitized mast cells, too, in response to the homologous antigen, both *in vivo* and *in vitro*. Now ample experimental evidence is available to prove the cell-damaging effect of various physical and chemical factors, as well as of the homologous antigen (on the sensitized mast cells).

Correspondingly, in mast cell research attention is now focussed on the problem of the disruptive action of various factors, on the storage and release of certain substances and whether the mast cells produce or merely store these substances.

Present knowledge in this field has been reached slowly, in spite of the fact that a survey of the literature of the past decades reveals many observations, most of them clinical, that fit into the pattern of our present views. Thus for example it was demonstrated by NETTLESHIP (1896), who was the first to describe urticaria pigmentosa, that in that disease the cutaneous lesions undergo swelling in response to mechanical stimulation (the intact skin of the patient is also inclined to urticaria factitia) and the adjacent skin shows erythema. UNNA (1896) showed by staining with alkaline methylene blue and safranine that in urticaria pigmentosa the cells occurring in heaps and having finely granulated cytoplasm, described already by THIN (1877), were actually mast cells, and the foci of urticaria pigmentosa could be considered to be mastocytomas. The reactions of these foci to mechanical stimulation, observed by the end of the past century (NETTLESHIP, THIN, UNNA) were identical with the triple response of LEWIS. BÄUMER studied mechanically stimulated foci of urticaria pigmentosa and areas of his own skin exposed to nettle stings on 4 consecutive days observed that some of the mast cells had disintegrated, degranulated, first of all in the marginal areas of the excised specimens (mechanical effect?); the granules that had left the cells were dispersed not only near the cells, but also some of the cells originating from proliferating adventitial cells contained metachromatically staining mast cell granules. It has been observed repeatedly in oedematous, i.e. mechanically stimulated, foci that the mast cells had disintegrated and their metachromatic granules and their remnants had scattered (BÄUMER, RAAB, KREIBICH, LEHNER). LEHNER (1926) in a case of urticaria pigmentosa found large numbers of mast cells which had lost their specific granules; the residues had massed mainly at the margin or at one pole of the cells, and fine granules were visible extracellularly. These findings must have originated from mechanically stimulated foci, at least this is indicated by the histologically demonstrated oedema. TÖRÖK (1928) ascribed the oedematous swelling caused by a mechanical stimulation of urticaria pigmentosa foci to the action of inflammatory agents released from the tissues and explained the diminution of local urticaria by claiming that the vascular irritants are not produced in such amounts as would suffice to produce urticarial swelling. As early

as 1899, BRONGERSMA attributed urticarial swelling to "toxic" substances, originating from such degenerated mast cells which had lost their granules. DALE (1929) attributed to local histamine release the reactions so readily elicitable by mechanical stimulation of the foci of urticaria pigmentosa.

The above data, representing interesting advances in the history of our knowledge of mast cells, can be fitted into the pattern of our present views, yet in fact no direct conclusions have been drawn from them. The mostly clinical observations have hardly been made use of in the research aimed at the elucidation of mast cell function. Even the general weakness so common in urticaria pigmentosa aroused no suspicion that histamine may be responsible for it, even after the biological histamine reactions and histamine shock had become known. After the discovery made by RILEY and WEST this possibility seems to be natural and for example BLOOM et al. (1958) speak about recurrent histamine shocks in the case of a child with urticaria pigmentosa.

RILEY and WEST could approach the relation between mast cells and tissue histamine from a new and more direct angle when MACINTOSH and PATON (1949) had shown that a wide variety of organic bases have specific histamine liberator activity; when injected into the organism, they give rise to typical histamine reaction, so for example on intravenous injection they produce in the dog symptoms resembling those of anaphylactic or peptone shock. RILEY and WEST have shown that the basic amines peptone and d-tubocurarine cause vacuolisation, degranulation and disintegration of the mast cells. The location within the mast cell of the liberator has been studied by using stilbamidine, or 2-hydroxystilbamidine, fluorescent histamine liberators readily observable under UV light, and it has been found that prior to the disruption of cells the liberator is bound to the granules. In subsequent works RILEY and WEST demonstrated the close correlation between the mast cell and the histamine contents of tissues by studying a very wide variety of intact and diseased tissues.

These data, together with some more recent ones, amply suffice to prove that mast cells actually store histamine. This was further confirmed by showing that other factors known to release histamine also damage the mast cells: distilled water (FAWCETT, 1954, 1955); freezing (CRAPS 1959); X-ray irradiation (SMITH, 1953 and 1954; BRENK, 1958); polymyxin-B (BUSHBY and GREEN, 1955); phospholipase-A (HÖGBERG and UVNÄS, 1957); heterologous protein (EDER and SCHAUER, 1961); Ascaris l. extract (HÖGBERG and UVNÄS, 1958); rat spleen extract (ARCHER, 1959); and, according to our own investigations, hypotonic salt solutions and distilled water (1944, 1962), heat (1944, 1962), colchicine and nitrogen mustard (1954), X-ray irradiation (1953), UV irradiation (1948, 1962), and heterologous serum (1962).

Moreover, it has been proved for several species of animals that in the course of anaphylactic or anaphylactoid reactions the mast cells suffer morpho-

logical and functional changes, depending in measure on the grade and character of sensitisation, on the amount of the antigen, its purity and mode of administration (JAQUES, 1941; STUART, 1952; MOTA, 1953; MOTA and DIAS DA SILVA, 1958, 1960; MOTA, 1956, 1959; MOTA and VUGMAN, 1956; HUMPHREY and MOTA, 1959; WEGELIUS, 1955; HÖGBERG and UVNÄS, 1958; UVNÄS and THON, 1959; HIGGINBOTHAM, 1959; KELLER, 1957; KELLER and SCHWARZ-SPECK, 1961; KELLER and BEEGER, 1961; TOKUDA, 1961; SALVATO, 1961; GRÓF, 1962).

Beside the relatively monotonous morphological changes in the mast cells (enlargement, vacuolisation, loosening of cell borders, partial or total degranulation, and ultimately total disruption) they undergo a wide range of functional changes. Of these, a few are only known, and their consequences cannot be restricted to the early vascular and tissue reactions caused by the released histamine or eventually by heparin and serotonin. The late and thus far little studied connective tissue reactions seem to be just as significant. The complexity of the function of mast cells is indicated also by the fact that they contain apart from heparin and histamine a variety of other substances, such as serotonin (BENDITT et al., 1955); SRS (Slow Reacting Substance) (BROCKLEHURST, 1960; CHAKRAVARTY, 1960; UVNÄS and THON, 1961); various kinds of lipids (RHEINGOLD, 1948; SCHAUER and EDER, 1961; HORVÁTH, 1959, 1960), and enzymes (LAGUNOFF and BENDITT, 1959; EDER and SCHAUER, 1960; MONTAGNA, 1956; BRAUN-FALCO, 1959).

According to ASBOE-HANSEN, ASTALDI and VELICAN the mast cells take part in the production of hyaluronic acid and, according to SYLVÉN, in that of the metachromatic ground substance of granulation tissue. RILEY (1959) has claimed that the mast cells or their metabolites influence the function of fibroblasts, the formation (neogenesis, absorption) of connective tissue fibres. HIGGINBOTHAM et al. have shown that the released mast cell granules are soon phagocyted by fibroblasts (micellophagosis) where they are demonstrable for a time by their metachromatic staining ("quasi-mast cells").

In the light of the above it is clear that in dermatology the data concerning the physiological role of mast cells are significant for explaining the mechanism of normergic and allergic inflammation, in addition to the well-known benign and malignant neoplasms associated with a pathological increase in the number of mast cells. It is therefore only natural that at the 1960 Dermatological Congress in Hamburg the problem of mast cell research was taken up among the main subjects with the cooperation of clinicians and research workers concerned with various aspects of the problem.

One of the principal conditions of mast cell research is that mast cells be available in sufficient quantities and possibly free from other cells. There are three methods to achieve this.

1. Isolation of mast cells from mixed-cell abdominal washings rich in

mast cells (rat) (PADAWER-GORDON, 1955; GLICK, 1956; ARCHER, 1959; KELLER, 1961; UVNÄS and THON, 1959).

2. Surviving peritoneum. This is easy to obtain and has the advantage of ensuring for a time approximately physiological conditions, at least for the purposes of acute experiments.

3. Use of mastocytomas, especially transplantable mouse mastocytoma, which may be considered to be practically pure mast cell cultures.

Experimental

In our studies we have made use of all three possibilities. In the present paper we shall report on the serial passage in albino mice of FURTH's transplantable mastocytoma and the morphological properties of the tumours thus obtained, as well as the histamine contents of the tumours and various organs (skin, brain, lung, intestine, liver, spleen, kidney) of the tumorous animals. In one case we determined the histamine contents in a LAF_1 mouse mastocytoma and some organs.

Methods

a) *Serial passage of transplantable mastocytoma in the albino mouse.* The mastocytoma described by FURTH *et al.* arose in LAF_1 mice and in such mice the tumour takes in about 100 per cent of the cases. According to our observations in a small number of LAF_1 mice, the intramuscularly inoculated tumour shows rapid local growth and produces leukaemia-like infiltrations in spleen and liver. We have been passaging the tumour by intramuscular injection in our own breed of albino mice since 1961. Prior to inoculation the mice are exposed to 200—400 r total body irradiation. In our animals the tumour showed exclusively local growth. We have made also intravenous and intraperitoneal inoculations and also in newborn animals. For passaging intramuscular tumours were used, excised under aseptic conditions, homogenised at 0.2—0.4 g/ml 0.9 per cent NaCl tissue concentration, then injected by means of a No. 12 Record cannula in amounts of from 0.1 to 0.4 ml/animal.

b) *Histologic and electron microscopic studies.* For studies under the light microscope the tumours were fixed in formalin, embedded in paraffin and stained with haematoxylin-eosin, periodic acid-Schiff, toluidine blue and GOMORI's silver impregnation. Also studied were specimens of liver, spleen, kidney, lung, heart and skin. For electron microscopic study, specimens 1 to 2 mm in diameter were excised from the tumours within 1 minute after killing the animal, fixed in 1 per cent, pH 7.2 buffered, isotonic osmium tetroxide, then dehydrated and embedded in methacrylate. The sections were cut by means of an LKB-Ultrotom ultramicrotome and studied in a Zeiss-ELMI-D_2 electron microscope, on microgrids covered with formvar membrane.

c) *Histamine assay* was made according to BARSOUM and GADDUM and CODE, on surviving guinea pig ileum, determining the total histamine content of the tumours, as well as that of various organs (brain, lung, small and large intestine, liver, spleen, kidney and skin, the latter taken from the proximity of the tumour and from the chest). The animals were killed in the 2nd or 3rd week following inoculations. The tumour, or the organ to be studied, was excised, weighed, homogenised, treated with 10 per cent trichloroacetic acid to extract histamine, and after several hours of extraction the extract obtained was analysed as specified.

Results

1. Passage of Furth's transplantable mastocytoma by intramuscular inoculation was successful in 10 to 15 per cent of adult, non-irradiated mice, and in 20 to 40 per cent of animals irradiated prior to inoculation *(Fig. 1)*.

The tumour killed the animals after 2 to 4 weeks of growth. Growth was local, partly invasive. Sometimes the tumour became ulcerated, but secondary deposits were never formed. Intravenous and intraperitoneal inoculations produced no tumorous growth in adult mice, while in newborn mice a growth mostly

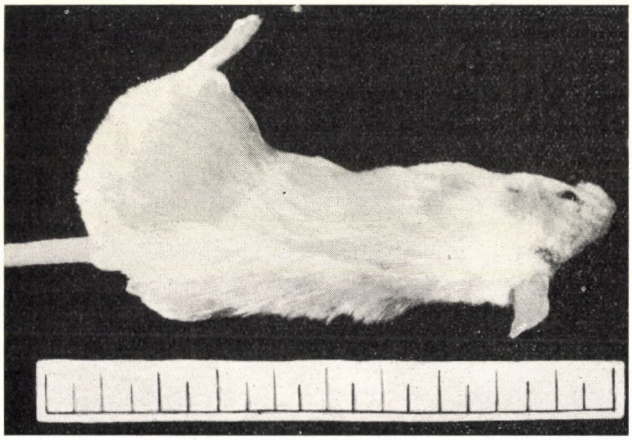


Fig. 1. Mastocytoma growing intramuscularly in left thigh (albino mouse, own breed)

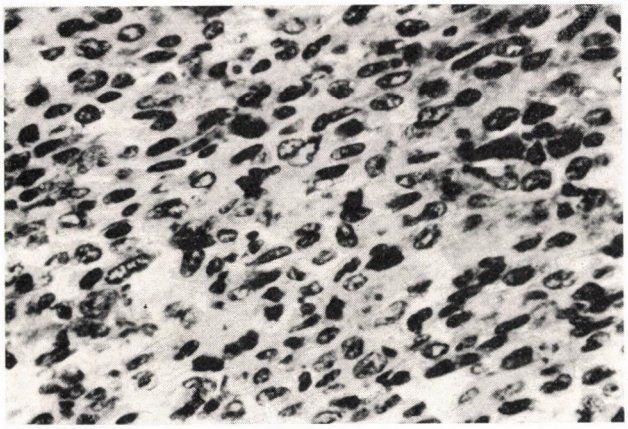


Fig. 2. Mastocytoma growing intramuscularly. Haematoxylin-eosin, about × 400 magnification

appeared following intraperitoneal inoculation, but these mice succumbed within a short time when the tumour was still small.

Under the light microscope, the richly cellular tumour tissue showed slight polymorphism and many mitoses *(Fig. 2)*. At sites the tumour cells were elongated, resembling fibroblasts. In the cytoplasm fine metachromatic and periodic acid-Schiff positive granulation could be seen. In the other organs of the tumorous animals no mastocytoma infiltrations could be detected.

2. Electron microscopic examination showed variable numbers of markedly osmiophilic granules, surrounded at sites by a distinct membrane (*Fig. 3*).

3. The results of histamine assay are presented in *Table I* and *Table II*.

a) In *Table I* is shown the histamine content in the tumour, the skin over it and the presumably intact skin taken from the chest. The cases are listed in the order of tumour weight.

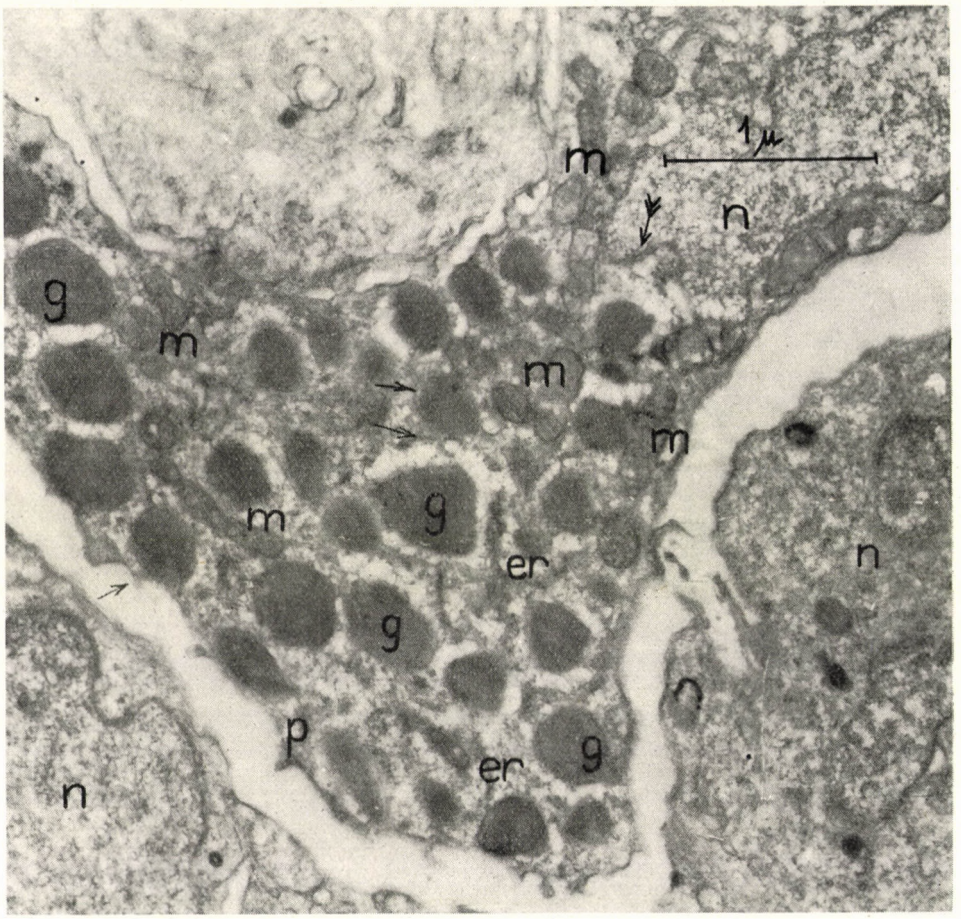


Fig. 3. Electron microscopic appearance of mastocytoma (fixation with osmium, embedding in methacrylate)

Signs:

m: mitochondrium
n: nucleus
g: granules
er: ergastoplasm
double arrow: double nuclear membrane
arrow: granule membrane
p: Palade's granules

b) *Table II* shows the histamine content of the organs of tumorous mice, in the order specified in *Table I.*

c) In one case we determined the histamine content in the mastocytoma and some organs of a LAF_1 mouse (*Table III*).

Table I

Histamine content of the tumour and skin of mastocytomatous mice
(Histamine dichloride μg/g fresh tissue)

Case No.	Tumour		Skin			
	weight	H content	over the tumour		from the chest	
			weight	H content	weight	H content
I.	2.64	1114.0	0.32	187.0	0.18	18.0
II.	2.10	286.0	—	—	0.29	7.0
III.	2.02	120.0	—	—	0.31	20.0
IV.	1.85	540.0	0.16	156.0	0.12	14.6
V.	1.08	460.0	0.22	15.0	0.10	15.0
VI.	0.86	930.0	0.40	500.0	0.19	10.0
VII.	0.81	870.0	0.20	6.0	0.28	7.0
VIII.	0.78	1000.0	0.10	70.0	0.22	25.0

Table II

Histamine content of the organs of mastocytomatous mice
(Histamine dichloride μg/g fresh tissue)

Mouse No.	Brain	Lung	Small intestine	Large intestine	Liver	Spleen	Kidney
I.	0.35	5.7	13.3	4.0	3.6	2.5	5.0
II.	0.5	2.0	12.2	2.3	—	4.5	0.25
IV.	0.45	5.5	2.3	2.1	1.0	2.0	3.5
V.	0.12	3.0	7.0	6.3	2.5	1.8	2.2
VI.	0.30	—	12.0	3.7	1.6	—	3.0
VII.	0.30	6.0	11.8	7.5	2.6	—	2.5
VIII.	—	—	6.0	5.5	3.8	6.3	7.3

Note: Mouse No. III was not tested for histamine content of the organs.

Table III

Histamine content of mastocytoma and some organs of an LAF_1 mouse
(Histamine dichloride μg/g fresh tissue)

Tissue tested	Tumour	Lung	Heart	Kidney	Liver
H content	500.0	120.0	50.0	40.0	1600.0

Discussion

1. We have succeeded in passaging Furth's transplantable mastocytoma in albino mice of our own breed. The frequency of the takes remained behind the almost 100 per cent observed in the LAF_1 mouse, but was sufficient for obtaining adequate quantities of mast cells for further investigations. The tumours grew locally, no secondary deposits were formed, no infiltrations in liver and spleen developed, indicating a lower susceptibility to mastocytoma of our mice than that of LAF_1 mice. The light and electron microscopic appearance of the tumour mast cells resembled those of normal, loose connective tissue, although the number of metachromatically staining granules was less in the former than in the latter. The cause of this is unknown, and further investigations are required to elucidate an eventual correlation of granule contents with the lower susceptibility to mastocytoma.

2. The histamine content of the mastocytomas induced by inoculation was high, similar to other types of mastocytoma, in accordance with the view put forward by RILEY and WEST. Considerable differences in histamine concentration were found between the single tumours. The highest value (1114.0 μg/g) was about tenfold the lowest one (120.0 μg/g). Histology revealed no such structural differences or shifts in the relative percentage of mast cells and other tissue elements as could have explained the differences in histamine content. The animals were of approximately the same age and were fed an identical diet.

However, more relevant evidence is obtained by comparing the age and development of the tumours with the histamine content. The data in *Table I* make it clear namely that the relative histamine content of the bigger, more developed, tumours weighing about 2 g was less than that of younger, smaller tumours. The relative histamine concentration in the 2.02 g tumour of case III was 120.0 μg/g, with the absolute amount of stored histamine as low as 240 μg. In contrast, in the smallest tumour, one weighing 0.78 g (case VIII), the relative histamine concentration was 1000.0 μg/g, and the absolute histamine content 780 μg. Although the highest relative and absolute histamine contents were found in the biggest tumour, and this is at variance with what we have just been elaborating, the combined evaluation of the eight tumours analysed indicates that the bigger, more developed tumours can store (produce?) histamine less efficiently than the younger, more actively growing ones. It is difficult to explain this phenomenon, because we found no substantial differences in tissue structure, in the proportion of tissue elements of the tumour. Since, however, it is exclusively in the mast cells that histamine is being stored or produced, the difference in histamine content shown by the tumours can be ascribed exclusively to changes in the ability of mast cells to produce or store histamine. This change in mastocytic activity can hardly be compared

with the physiological change of just the opposite direction, in the course of which the mast cell and histamine content of the same organ change with age; in the organs of foetal or very young mammals the mast cell and histamine contents are less than in the same organs of the adult animals (RILEY, 1959). We prefer to ascribe the phenomenon to the well-known differences in activity between the peripheral and central parts of the tumour (connected with the metabolism of the neoplasm), and, correspondingly, it might be suggested that the mast cells of the young, growing tumour produce more histamine. This possibility seems to fit well into the pattern of recent information concerning histamine production by mast cells. By using C^{14}-labelled histidine, SCHAYER showed that in the peritoneal mast cells of the rat, or in the blood platelets of the rabbit under the effect of histidine-decarboxylase histamine is formed from histidine. This histamine is stably linked at the site of its formation. Later (1961, 1962), the same author has shown that in the cells the enzyme is present in "inducible" form and in response to ambient effects its activity changes so that the production of histamine required for the regulation of local blood supply is always ensured.

Otherwise, there is scarce information concerning the histamine content of the mouse mastocytoma. SJOERDSMA et al. (1957) found in Dunn-Potter tumour 470 to 560 μg/g, FURTH et al. (1957) in Furth tumour 0.85 to 4.2 mg/g, and CASS et al. (1958) in lyophylised mastocytoma material 1700 μg/g, of histamine.

3. In the majority of cases the skin specimens taken from the tumorous area were rich in histamine. In one case (No. VI) the content was of the same order of magnitude as in the tumour. This tends to indicate that the tumour has already infiltrated the skin, and the skin contained large masses of mast cells. In the skin surrounding the tumour the relative proportion of mast cells to the skin's own elements was much lower than in the tumour itself. The high histamine content of the skin might be due to a relatively higher activity, to a higher histamine production of the mast cells, just like in the case of young tumours. (This means that, in accordance with the hypothesis of SCHAYER, here, too, an increased histidine-decarboxylase activity may be involved, but this assumption necessarily means that in further work the various areas of mastocytomas should be tested for histidine-histamine content and for histidine-decarboxylase activity.)

4. In the skin of the chest the histamine content was at the physiological level. The values obtained by us, even the highest one (case VIII: 25.0 μg/g), were much lower than the 40 μg/g value determined by PERRY in five animals. The histamine content was never high in the other organs of tumorous mice. For the time being it is impossible to compare our results with physiological data, as these are lacking in the literature; we have made no such determinations, either. The values obtained were closely similar to the physiological ones

described in the literature and found also by us in the rat, both as regards the order of magnitude of the histamine level and the relative histamine concentrations in the organs. As the histamine values found in the organs of our mastocytomatous mice can be regarded as physiological, it seems likely that on the basis of the histamine content, in our tumorous animals the mast cell content of the organs has not increased, *i.e.* in our mouse strain the transplantable mastocytoma grew exclusively locally and formed secondary deposits in none of the organs. Thus, our mice are less susceptible to mastocytoma than are the LAF_1 mice.

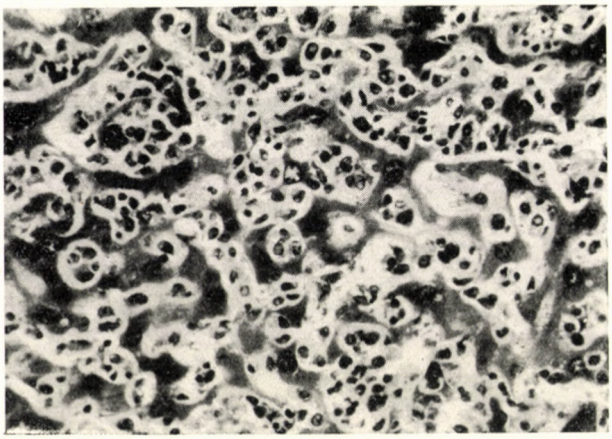


Fig. 4. Liver of a mastocytomatous LAF_1 mouse. Haematoxylin-eosin, about × 400. Masses of mastocytomatous infiltrations are visible in the hepatic sinusoids

5. In contrast with this, every organ of the original LAF_1 mouse *(Table III)* contained more histamine than the organs of our mice, and in the liver excessively high amounts were found. These data comply fully with the observation that in LAF_1 mice the intramuscularly inoculated tumour not only shows rapid local growth, but also forms leukaemia-like secondary deposits in the organs. Parallel with the increase in the number of mast cells caused by metastasis formation, the histamine content increased manifold. The 1600 μg/g histamine content found in the liver was much higher than that in the tumour itself. Although there was a massive mast cell infiltration in the liver *(Fig. 4)*, the excessively high histamine content may also be correlated with the metabolism of that organ. In the liver namely the preconditions of excessive histamine formation (histidine, histidine-decarboxylase activity) are better than in the mastocytoma itself.

In connexion with the capacity of mastocytomas to produce histamine let us mention that LINDELL et al. (1959) studied with C^{14}-L-histidine the histamine producing capacity of a mastocytoma located in the dog's abdominal

skin, and found that in the central part of the tumour extremely rich in mast cells histamine production was 4 to 4.5 times greater than in the subcutaneous tissue adjacent to the tumour. From their report it cannot be established whether the subcutis was intact or infiltrated with mast cells.

Acknowledgements

We are indebted to Miss Anna B. Cseh and Miss Klára R. Tóth for technical assistance.

REFERENCES

ARCHER, G. T.: a) Nature (Lond.) **182,** 726 (1958); b) Nature (Lond.) **184,** 1151 (1959); c) Aust. J. exp. Biol. med. Sci. **37,** 383 (1959).
ASBOE-HANSEN, G.: Acta derm.-venereol. (Stockh.) **30,** 338 (1950).
ASTALDI, G., RONDANELLI, E. G., BERNADELLI, E.: Rev. Hémat. **8,** 105 (1953).
BALI, T., FURTH, J.: Amer. J. Pathol. **25,** 605 (1949).
BARSOUM, G. S., GADDUM, J. H.: J. Physiol. (Lond.) **85,** 1 (1935).
BÄUMER, E.: Arch. Derm. Syph. (Berl.) **34,** 323 (1896).
BENDITT, E. P., WONG, A. L., ARASE, M., ROEPER, E.: Proc. Soc. exp. Biol. (N. Y.) **90,** 303 (1955).
BENDITT, E. P., KOBAYASHI, I.: Proc. Soc. exp. Biol. (N. Y.) **92,** 653 (1956).
BHATTACHARYA, B. K., LEWIS, G. P.: Brit. J. Pharmacol. **11,** 202 and 411 (1956).
BLOOM, G., DUNÉR, H., PERNOW, B., WINBERG, J., ZETTERSTRÖM, R.: Acta paediat. (Uppsala) **47,** 152 (1958).
BRAUN-FALCO, O., SALFELD, K.: Virchows Arch. path. Anat. **303,** 325 (1929).
BRAUN-FALCO, O., SALFELD, K.: Nature (Lond.) **183,** 51 (1959).
BRENK, H.: Brit. J. exp. Path. **39,** 356 (1958).
BROCKLEHURST, W.: J. Physiol. (Lond.) **151,** 416 (1960).
BRONGERSMA, H.: Brit. J. Derm. **11,** 179 (1899).
BUSHBY, S. R. M., GREEN, A. F.: Brit. J. Pharmacol. **10,** 215 (1955).
CASS, R., MARSHALL, P. B., RILEY, J. F.: J. Physiol. (Lond.) **141,** 510 (1958).
CHAKRAVARTY, N.: Acta physiol. scand. **48,** 167 (1960).
CODE, C. F.: J. Physiol. (Lond.) **89,** 257 (1937).
CRAPS, L.: a) Arch. belg. Derm. Syph. **15,** 119 (1959); b) Arch. klin. exp. Derm. **213,** 582 (1961).
DALE, H. H.: Lancet **1,** 1233 and 1285 (1929).
DUNN, T. B., POTTER, M.: J. nat. Cancer Inst. **18,** 587 (1957).
EDER, M.: Verh. dtsch. Ges. Path. **42,** 374 (1958).
EDER, M., SCHAUER, A.: Beitr. path. Anat. **124,** 251 (1961).
EHRLICH, P.: Arch. mikr. Anat. **13,** 263 (1877).
FAWCET, D. V.: a) J. exp. Med. **100,** 217 (1954); b) Anat. Rec. **121,** 29 (1955).
FURTH, J., HAGEN, P., HIRSCH, E. I.: Proc. Soc. exp. Biol. (N. Y.) **95,** 824 (1957).
GLICK, D., BONTING, S. L., BOER, D. D.: Proc. Soc. exp. Biol. **92,** 357 (1956).
GRÓF, P.: a) Dermatologica, **90,** 105 (1944); b) Bőrgyógy. Vener. Szle. **39,** 41 (1963).
HIGGINBOTHAM, R. D.: Int. Arch. Allergy. **15,** 195 (1959).
HORVÁTH, L.: Acta morph. Acad. Sci. hung. **9,** 35 and 179 (1960).
HÖGBERG, B., UVNÄS, B.: a) Acta physiol. scand. **41,** 345 (1957); b) Ibid. **44,** 157 (1958); c) Ibid. **45,** 255 (1959).
HUMPHREY, I. H., MOTA, I.: Immunology, **2,** 31 (1959).
JAQUES, L. B., WATER, E. T.: J. Physiol. (Lond.) **99,** 454 (1941).
JORPES, J. E., HOLMGREN, H., WILANDER, O.: Z. mikr. anat. Forsch. **42,** 279 (1937).
KELÉNYI, G.: a) Acta morph. Acad. Sci. hung. **3,** 377 (1953); b) Ibid. **4,** 345 (1954).
KELLER, R.: a) Int. Arch. Allergy. **11,** 328 (1957); b) Helv. physiol. pharmacol. Acta **19,** C27 (1961); c) Experientia (Basel) **18,** 286 (1962).
KELLER, R., SCHWARZ-SPECK, M.: Schweiz. med. Wschr. **91,** 1196 (1961).
KELLER, R., BEEGER, I.: Med. exp. (Basel) **4,** 51 (1961).
KREIBICH, K.: Arch. Derm. Syph. **48,** 163 (1899).
LAGUNOFF, D., BENDITT, E. P.: a) J. Histochem. Cytochem. **7,** 286 (1959); b) Amer. J. Physiol. **196,** 993 (1959).
LEHNER, I.: Dermat. Z. **46,** 87 (1926).

LINDELL, S. E., RORSMAN, H., WESTLING, H.: Experientia (Basel) **15,** 31 (1959).
MCINTOSH, F. C., PATON, W. D. M.: J. Physiol. (Lond.) **109,** 190 (1949).
MICHELS, N. A.: The Mast Cells. in Handbook of Haematology. Hoeber, New York 1938. Vol. I. pp. 232—354.
MONTAGNA, W.: The Structure and Function of Skin. Academic Press, New York 1956.
MONTAGNA, W., EISEN, A. Z., GOLDMAN, A. S.: Quart. J. micr. Sci. **95,** 1 (1954).
MOTA, I.: a) Thesis, University of S. Paulo (1953); b) Brit. J. Pharmac. **12,** 453 (1957); c) J. Physiol. (Lond.) **147,** 425 (1959).
MOTA, I., WUGMAN, I.: Nature (Lond.) **177,** 427 (1956).
MOTA, I., DIAS DA SILVA, W.: Nature (Lond.) **185,** 245 (1960).
NETTLESHIP, E.: Brit. med. J. **2,** 323 (1869).
NIEBAUER, G.: a) Wien. klin. Wschr. **68,** 447 (1956); b) Acta neuroveg. (Wien) **18,** 297(1958); c) Acta histochem. (Jena) Suppl. **1,** 260 (1958); d) Arch. klin. exp. Derm. **213,** 556 (1961).
PADAWER, J., GORDON, A. S.: Proc. Soc. exp. Biol. (N. Y.) **88,** 29 (1955).
PERRY, W.: in Ciba Symposium on Histamine, Churchill, London (1956) P. 242.
RAAB, W.: Festschrift Kaposi, 1900; Ref.: Mh. prakt. Derm. **34,** 195 (1902).
RAAB, W.: a) Arch. klin. exp. Derm. **214,** 307 (1962); b) Ibid. **215,** 1 (1962).
RHEINGOLD, J., WISLOCKI, G. B.: Blood, **3,** 641 (1948).
RILEY, J. F.: a) J. Path. Bact. **65,** 461 (1953); b) Ibid. **65,** 471 (1953); c) The Mast Cells, Livingstone, Edinburgh (1959).
RILEY, J. F., WEST, G. B.: a) J. Physiol. (Lond.) **120,** 528 (1953); b) J. Path. Bact. **69,** 269 (1955); c) Arch. int. Pharmacodyn. **102,** 304 (1955).
SALVATO, G.: Int. Arch. Allergy, **18,** 348 (1961).
SCHAUER, A., EDER, M.: Klin. Wschr. **39,** 76 (1961).
SCHAYER, R. W.: Amer. J. Physiol. **186,** 199 (1956).
SCHAYER, R. W.: Proc. XXII. Int. Physiol. Congr. (Leiden), 1962. p. 852.
SCHAYER, R. W., KOBAYASHI, I.: Proc. Soc. exp. Biol. (N. Y.) **92,** 653 (1956).
SJOERDSMA, A., WAALKES, T. P., WEISSBACH, H.: Science **125,** 1202 (1957).
SMITH, L. E., LEWIS, Y. S.: a) Proc. Soc. exp. Biol. (N. Y.) **82,** 208 (1953); b) Ibid. **85,** 306(1954).
STUART, E. G.: Anat. Rec. **112,** 394 (1952).
SYLVÉN, B.: in Symposium on Connective Tissue. Ed. Tunbridge, R. E. Blackwell, Oxford 1957, P. 27.
THIN, G.: Trans. clin. Soc. Lond. **10,** 198 (1877).
TOKUDA, S.: J. Immunology, **79,** 259 (1957).
TÖRÖK, L.: in Handbuch der Haut- und Geschlechtskrankheiten, Springer, Berlin 1928, Vol. VI/2.
UNNA, P. G.: Histopathologie, Berlin 1894, p. 964.
UVNÄS, B., THON, I. L.: a) Exp. Cell Res. **18,** 512 (1959); b) Ibid. **23,** 45 (1961).
UVNÄS, B., HÖGBERG, B.: Acta physiol. scand. **44,** 157 (1958).
VELICAN, C., VELICAN, D.: Acta haemat. (Lpz.) **21,** 108 (1959).
WEGELIUS, O., HJELMAN, G., WASASTJERNA, C.: Acta path. microbiol. scand. **36,** 309 (1955).
WEST, G. B.: in Ciba Symposion on Histamine. Churchill, London 1956.

Dr. Pál GRÓF
Dr. Gábor KELÉNYI
Orvostudományi Egyetem, Pécs, Hungary

THE EFFECT OF NORADRENALINE ON CARDIAC FLOW AND PERFORMANCE IN ISCHAEMIC SHOCK

By

GY. SZABÓ and SUSAN MAGYAR

INSTITUTE OF TRAUMATOLOGY, BUDAPEST (DIRECTOR: PROF. GY. SZÁNTÓ) AND FIRST DEPARTMENT OF MEDICINE (DIRECTOR: PROF. I. RUSZNYÁK), UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received May 10, 1963)

In dogs with ischaemic shock cardiac output was found to decrease approximately parallel with blood pressure. Coronary blood flow decreased to a lesser extent. Coronary resistance showed no significant alteration.

Infusion of noradrenaline restored arterial pressure, without a significant increase in cardiac output; the rise in pressure was due to an increase in peripheral resistance. Coronary resistance decreased rather than increased. Accordingly, coronary blood flow rose above control values, and the coronary fraction of the cardiac output increased substantially.

Traumatic shock is characterized by heamodynamic changes, of which the most important are a significant decrease in cardiac output and arterial blood pressure, with consequent impairment of the blood supply to the vital organs.

It was almost half a century ago that coronary circulation has been established to depend on arterial pressure [26, 28]. This dependence has since been confirmed by a host of authors [6, 20, 21, 23]. Congruently, coronary circulation has been demonstrated to decrease also in haemorrhagic hypotension [3, 7, 12, 29, 30], yet HACKEL and GOODALE [19] found no essential change in cardiac blood flow because of a concurrent decrease in coronary resistance.

Coronary circulation has been shown to decrease in traumatic shock. This decrease, however, is not proportional to the fall in blood pressure, which fact indicates dilatation of the coronary vessels [39]. SARNOFF et al. [30], on the other hand, observed in the late stages of traumatic shock a marked decrease in coronary flow; however, an elevation of the perfusion pressure definitely improved the blood supply.

Some of the treatments currently employed serve the immediate end of raising blood pressure to improve blood supply to the vital organs. Although in the light of recent experimental findings the use of pressor substances for this purpose seems somewhat questionable, clinical studies [e.g., 27] have shown that unless the cause of shock can be removed at once, early restoration to normal of blood pressure by pressor treatment is favourable. Some authors [19, 24] claim that noradrenaline exerts a favourable action in haemorrhagic shock, even if administered in addition to adequate blood transfusions. This

action may perhaps be questioned in haemorrhagic shock, but in clinical literature there is agreement in that noradrenaline is eminently suitable for the control of other types of shock, primarily that due to coronary occlusion [37].

Studied in a variety of preparations (fibrillating heart, heart-lung preparation, heart perfused in situ), both adrenaline and noradrenaline were found definitely to increase coronary flow [6, 8, 9, 10, 17, 22, 26], to improve cardiac flow in experimental coronary occlusion [35], and to promote myocardial oxygenation [31]. There are, however, no reliable data as to the changes in blood flow through the vital organs, including the heart, when some pressor agent is administered for restoring blood pressure during shock. CATCHPOLE et al. [3] and FRANK et al. [13] found that noradrenaline greatly intensified coronary flow during haemorrhagic hypotension, but the question is still open as to what extent the values obtained in this particular shock pattern apply to the various forms of shock. The perfusion of the vital organs with respect to the efficacy of pressor treatment being apparently imperative, we decided to study it in experimental ischaemic shock.

Pressor agents raise blood pressure by inducing peripheral vasoconstriction whereby they increase circulatory resistance and the work of the heart. On this ground it seems possible that relative myocardial ischaemia is aggravated despite an accelerated coronary flow. On this account we thought it necessary to study systemic circulation and cardiac work, and to compare their changes with those in cardiac flow.

Material and methods

In ten mongrel dogs of 15 to 20 kg body weight ischaemic shock was induced under chloralose anaesthesia (0.10 g/kg administered intravenously) by applying a tourniquet on the two hind legs for 4 hours. In previous experiments [33, 34] the method was certain to cause fatal shock. To measure coronary circulation a rotameter type flowmeter was inserted through a polythene cannula into the descending branch of the left coronary artery, and the system was perfused from the ipsilateral carotid artery. This time we departed from our earlier method [36] in that after connection of the flowmeter we closed the thorax and eliminated the pneumothorax so that the animals could breathe spontaneously. Cardiac output was estimated by the direct Fick method. Mixed venous blood was obtained through a catheter inserted into the right atrium, and arterial blood from the carotid artery. In the carotid also was measured the arterial pressure, by means of a mercury manometer. From the data obtained were calculated the total peripheral resistance (TPR), the coronary resistance (CR), the work of the left heart (CL), the coronary fraction of cardiac output (C_f), and the cardiac O_2 supply (AO_2), by means of the formulas,

$$\underset{(\text{dyn. sec. cm}^{-5})}{\text{TPR}} = \frac{M \times 1332}{\frac{V}{60}}$$

where M = mean arterial pressure, and V = cardiac output;

$$\underset{(\text{dyn. sec. cm}^{-5})}{\text{CR}} = \frac{M \times 1332}{\frac{Vc}{60}}$$

where Vc = blood flow through the left coronary artery, ml/min;

$$\underset{\text{(kg/min.)}}{\text{CL}} = \frac{M \times V \times 13.2}{1000}$$

where the kinetic factor is neglected; and

$$C_f = Vc/V \times 100$$

where it must be taken into consideration that only the amount of blood flowing through a single coronary branch, and not through the entire vascular system of the heart, was measured. From this it follows that the coronary fraction thus computed permits of relative evaluation only.

Cardiac oxygen supply was calculated from the product of coronary flow and arterial O_2 content.

For technical reasons, the first analysis was made at the end of the 4-hour ischaemia of the hind legs, before removing the tourniquets. Although the haemodynamic values determined on this occasion varied within normal limits, they represent but relative control values, which can only be related to the changes observed subsequently. After removing the tourniquets we were waiting for the blood pressure to drop to about half the initial value, or to approximately 60 mmHg, and then started an intravenous drop infusion of noradrenaline. The rate of infusion was so regulated as to achieve stabilization of blood pressure at the control level registered immediately before the removal of the tourniquet. In most cases this required administration of large noradrenaline doses, up to 10 γ/kg/min. Infusion was usually stopped when the animal had received a total of 5 mg of noradrenaline. Registrations were made on four occasions during the experiment, (1) immediately before removing the tourniquet; (2) when blood pressure fell to about 60 mmHg, before noradrenaline infusion was started; (3) during noradrenaline infusion; (4) as soon after completed infusion as blood pressure had stabilized at a low level.

Results

As mentioned above, we have no values that could be regarded as "normal" in the strict sense of the term, but the mean arterial pressure of the animals was normal at the end of the ischaemic period (123 mmHg), and so was the cardiac output. Despite a slight tachycardia (an average pulse rate of 131, somewhat high for animals weighing 15 to 20 kg), no essential haemodynamic changes were noted. Nor did the value for coronary flow (39.5 ml/min) differ from that obtained in other experiments carried out with similar methods [35, 36].

During the hypotensive period following removal of the tourniquet (average arterial pressure about 61 mmHg), there occurred a marked decrease in cardiac output, amounting to about 50 per cent; total peripheral resistance remained unchanged. Accordingly, the work of the heart diminished to about a quarter of what it had been before, whereas on the average coronary circulation decreased by not more than 37 per cent. Coronary resistance showed no significant change.

Upon the action of noradrenaline, blood pressure rose to the control level and cardiac output remained unchanged. This means that the rise in arterial pressure was due to an increase in peripheral resistance. This, however, did not extend to the coronary vessels, for coronary flow increased to two and a half times the pre-infusion value. The cause of this was first of all a rise in

Table 1

Haemodynamic effects of noradrenaline in ischaemic shock

		1	2	3	4
Blood press. mmHg	$\bar{x}$	123.1	61.3	123.3	45.3
n = 10	s.d.	21.6	10.9	18.8	17.4
Heart rate	$\bar{x}$	131.5	137.1	120.1	124.7
n = 8	s.d.	16.4	18.5	22.3	45.7
Card. output l/min.	$\bar{x}$	2.59	1.25^{++}	1.19^{++}	1.44
n = 8	s.d.	0.93	0.47	0.49	1.05
Periph. resist. dyn. sec. cm.$^{-5} \cdot 10^3$	$\bar{x}$	4.2	4.7	9.8^{+}	3.8
n = 8	s.d.	1.3	2.8	5.2	2.2
Cardiac work kg/min.	$\bar{x}$	4.57	1.04^{++}	1.94^{++}!	0.83!$^{+++}$
n = 8	s.d.	2.41	0.34	0.80	0.51
Coron. blood flow ml/min	$\bar{x}$	39.5	23.7^{+}	64.1^{+}!	17.1 !
n = 10	s.d.	20.8	20.5	44.1	16.0
Coron. resist. dyn. sec. cm$^{-5} \cdot 10^5$	$\bar{x}$	3.1	3.5	2.5 !	2.8
n = 10	s.d.	2.5	3.2	2.3	2.2
Coron. fraction Vc/V $\times$ 100	$\bar{x}$	1.47	1.38	4.77^{++}	1.40
n = 8	s.d.	0.86	1.17	2.03	0.53

$^{+}$: significant difference from control value at the 1% to 5% level
$^{++}$: significant difference from the control value at the 0.1% level
!: significant difference to the preceding period at the 1% to 5% level

1: Control period (before removal of tourniquet)
2: Before noradrenaline infusion
3: During noradrenaline infusion
4: After noradrenaline infusion

the perfusion pressure, but there was also a decrease in coronary resistance. The coronary fraction of the cardiac output increased substantially: whereas in the control period it was 1.5 per cent in the studied coronary branch, after noradrenaline it increased more than three and a half times, to 4.8. Owing to the increase in peripheral resistance the work of the heart increased to almost double of what it had been during the hypotensive period, but even so it failed to reach half its value registered in the control period at an identical

pressure. As no major changes occurred in arterial oxygen saturation, myocardial oxygen supply was roughly parallel with coronary flow.

Immediately after termination of the noradrenaline infusion the blood pressure fell steeply, to stabilize mostly at a lower than the pre-noradrenaline level. The cause of the steep fall was a decrease in peripheral resistance, or, in other words, the cessation of vasoconstriction. Coronary flow decreased together with arterial pressure, as in coronary resistance no significant change occurred at that point of time.

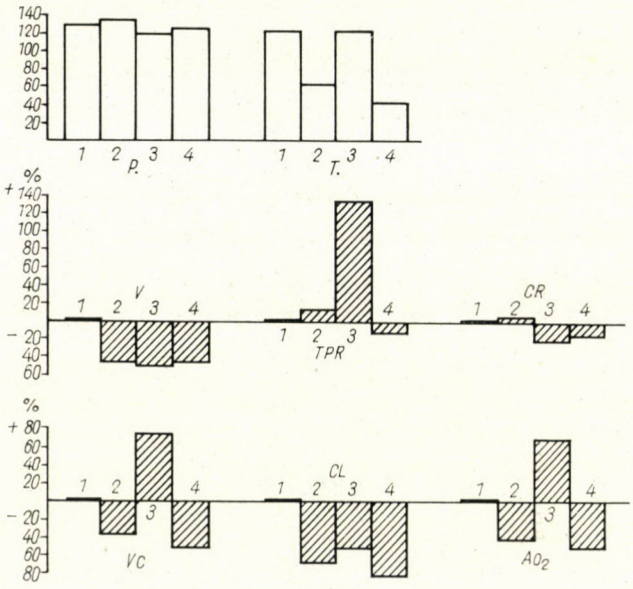


Fig. 1. Effect of noradrenaline in ischaemic shock

P = pulse rate
T = arterial pressure (mmHg)
V = changes in cardiac output (control period = 100%)
TPR = changes in total peripheral resistance
CR = changes in coronary resistance
Vc = percentage changes in coronary blood flow
CL = percentage changes in work of heart
AO_2 = changes in oxygen supply to coronary artery

Discussion

In our experiments characteristic haemodynamic changes have been observed in the early stage of ischaemic shock. Thirty to 60 minutes after the removal of the tourniquet arterial pressure fell steeply to about 60 mmHg, approximately half the initial value. The cause of the fall was a decrease in cardiac output. Total peripheral resistance remained unchanged.

Formerly, peripheral vasoconstriction had been considered a decisive factor in the shock mechanism [12, 14, etc.]. However, in conditions of shock vasoconstriction could be demonstrated in a few organs only, and studies of total vascular resistance failed to yield uniform results. This then induced WIGGERS and MIDDLETON [41] to distinguish four different types of peripheral resistance changes. The changes in peripheral resistance are, however, not characteristic, and there is usually no considerable increase in resistance. Even in traumatic shock such an increase occurs only in the late stage [38].

In our experiments coronary circulation decreased parallel with blood pressure. This points to the absence of a compensating mechanism which, at the expense of all the other organs, would ensure a preferential cardiac flow, and there was no significant change in the coronary fraction of cardiac output. It seems probable, however, that despite the decreased flow cardiac blood supply remained adequate as the supply decreased by 37 per cent when the work performed by the heart fell to about one quarter of what it had been before.

The stimulus producing coronary dilatation is in all probability not a decrease in blood flow or in arterial oxygen tension, but the myocardial oxygen requirement [6, 11, 38]. The latter depends of course on the work performed by the heart; moreover, on whether the alteration in that work is due to a change in resistance or in cardiac output [2]. No coronary dilatation is thus observable in the early stage of ischaemic shock, probably because the blood flow is sufficient to supply the oxygen required for the diminished work the heart now has to perform. In later stages, at a low blood pressure, severe myocardial hypoxia must arise, impairing the action of the heart [18].

Under the effect of noradrenaline, blood pressure was restored practically completely. For this, an increase of peripheral resistance was solely responsible, since no change was noted in cardiac output. At the same time, there was no increase in coronary resistance.

It is still a debated point whether noradrenaline increases coronary circulation passively, merely by raising perfusion pressure and *via* a vagal depression of the heart rate, or whether it actively dilates the coronary vessels, as had been postulated by SMITH et al. [32] and observed *in vitro* by DENISON et al. [4]. This issue cannot be solved on the basis of our present results but, considered from the practical side, this is of secondary importance. The essential point is that during noradrenaline infusion the work performed by the heart was less than half of what it had been in the control period, and that coronary blood flow and the oxygen supply to the heart exceeded control values. According to GOLLWITZER-MEIER and KROETZ [16], in judging whether or not coronary circulation meets the requirements one should not be guided by the relationship of work to blood flow but of oxygen consumption and cardiac work. Even if one accepted this conception, it would still seem

improbable that in conditions of shock noradrenaline should impair oxygen supply as it improves the flow without a parallel rise of cardiac work.

In the normal animal noradrenaline definitely improves cardiac oxygen supply since it decreases oxygen desaturation of the coronary sinus blood [25] and increases oxygen tension in the myocardium [31].

The conclusion to be drawn from these considerations is that in experimental ischaemic shock noradrenaline, by increasing peripheral resistance, brings about a redistribution of cardiac output, which in turn increases coronary blood flow and augments the coronary fraction of the cardiac output.

REFERENCES

1. Allela, A., Williams, F. L., Bolene-Williams, C., Katz, L. N.: Amer. J. Physiol. **183,** 570 (1955).
2. Braunwald, E., Sarnoff, S. J., Case, R. B., Sainsby, W. N., Welch, G. H.: Amer. J. Physiol. **192,** 157 (1958).
3. Catchpole, B. N., Hackel, S. B., Simeone, F. A.: Ann. Surg. **142,** 372 (1955).
4. Denison, A. B., Bardhanabaedya, A., Green, H. D.: Circulat. Res. **6,** 657 (1957).
5. Eckenhoff, J. E., Hafkenschiel, J. H., Landmesser, C. M.: Amer. J. Physiol. **148,** 582 (1947).
6. Eckenhoff, J. E., Hafkenschiel, J. H., Landmesser, C. M., Harmel, M.: Amer. J. Physiol. **149,** 639 (1947).
7. Edwards, W. S., Siegel, A., Bing, R. J.: J. clin. Invest. **33,** 1646 (1954).
8. Essex, H. E., Herrick, J. F., Baldes, E. J., Mann, F. C.: Amer. J. Physiol. **117,** 271 (1936).
9. Essex, H. E., Wégria, R., Herrick, J. F., Mann, F. C.: Amer. Heart J. **19,** 554 (1940).
10. Folkow, B., Frost, J., Uvnäs, B.: Acta physiol. scand. **17,** 201 (1949).
11. Foltz, E. L., Page, R. G.. Sheldon, W. F., Wong, S. K., Tuddenham, W. J., Weiss, A. J.: Amer. J. Physiol. **162,** 524 (1950).
12. Fozzard, H. A., Gilmore, J. P.: Naval Med. Field Res. Lab. Rep. **8,** 89 (1958).
13. Frank, E. D., Frank, H. A., Jacob, S., Weizel, H. A. E., Korman, H., Fine, J.: Amer. J. Physiol. **186,** 74 (1956).
14. Freeman, N. E.: Amer. J. Physiol. **103,** 185 (1933).
15. Gesell, R.: Amer. J. Physiol. **47,** 468 (1919).
16. Gollwitzer-Meier, K., Kroetz, Ch.: Pflügers Arch. Ges. Physiol. **240,** 248 (1938).
17. Green, H. D., Wégria, R., Boyer, N. H.: J. Pharmacol. **76,** 378 (1942).
18. Guyton, A. C., Criwell, J. W.: Fed. Proc. **20,** Suppl. 9, 51 (1961).
19. Hackel, D. B., Goodale, W. T.: Circulation **11,** 628 (1955).
20. Hausener, D. B., Essex, H. E., Herrick, J. F., Baldes, E. J.: Amer. J. Physiol. **131,** 43 (1940).
21. Hochrein, M., Keller, C. J.: Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. **159,** 300 (1931).
22. Katz, L. N., Lindner, E., Weinstein, W., Abramson, D. I., Joachim, K.: Arch. int. Pharmacodyn. **59,** 399 (1938).
23. Kimura, E., Kanazawa, T., Suzuki, N., Ito, Y., Haragai, N., Yamamoto, F.: Tohoku J. exp. Med. **66,** 33 (1957).
24. Lansing, A. M., Stevenson, J. A. F., Godwey, C. M.: Canad. J. biochem. Physiol. **35,** 93 (1957).
25. Lochner, W., Mercker, H., Schürmeyer, E.: Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. **227,** 373 (1956).
26. Markwalder, J., Starling, E. H.: J. Physiol. (Lond.) **47,** 275 (1913).
27. Mills, L. C., Voudoulis, I. J., Moyer, J. H., Heider, C.: Arch. intern. Med. **106,** 816 (1960).
28. Morawitz, P., Zahn, A.: Dtsch. Arch. klin. Med. **116,** 344 (1914).
29. Opdyke, D. F., Foreman, R. C.: Amer. J. Physiol. **148,** 726 (1947).
30. Sarnoff, S. J., Case, R. B., Waithe, P. E., Isaacs, J. P.: Amer. J. Physiol. **176,** 439 (1954).
31. Saven, J. J., Sheldon, W. F., Kuo, P. T., Zinssen, H. F., Horovitz, O., Sumen, A. F.: J. clin. Invest. **31,** 658 (1952).

32. SMITH, D. J., SYVERTON, J. T., COXE, J. W.: Circulation **4**, 895 (1951).
33. SZABÓ, GY., FEKETE, GY., RENNER, A.: In press.
34. SZABÓ, GY., MAGYAR, ZS.: Acta med. Acad. Sci. hung. **8**, 287 (1955).
35. SZABÓ, GY., SOLTI, F., MAGYAR, ZS.: Z. Kreislaufforsch. **47**, 24 (1958).
36. SZABÓ, GY., SOLTI, F., MAGYAR, ZS.: Z. Kreislaufforsch. **47**, 971 (1958).
37. TAINTNER, M. L.: Bol. Asoc. med. P. Rico **47**, 305 (1955).
38. TAKÁCS, L.: Acta physiol. Acad. Sci. hung. **11**, 55 (1957).
39. TAKÁCS, L., KÁLLAY, K., SKOLNIK, J.: Acta med. Acad. Sci. hung. **14**, 407 (1959).
40. WIGGERS, C. J.: Physiology of Shock. Commonwealth Fund, New York 1950.
41. WIGGERS, H. C., MIDDLETON, S.: Amer. J. Physiol. **140**, 677 (1944).

Dr. György SZABÓ
Dr. Susan MAGYAR
Budapest VIII. Mező Imre út 15—17., Hungary

CHROMOSOMENANOMALIE BEI SICH ZU LUPUS ERYTHEMATOSUS GESELLENDEM HÄMOLYTISCHEN IKTERUS

Von

S. NAGY

II. MEDIZINISCHE KLINIK (DIREKTOR: PROF. DR. GY. PETRÁNYI) DER MEDIZINISCHEN UNIVERSITÄT DEBRECEN

(Eingegangen am 13. Mai 1963)

Bei einem mit hämolytischem Ikterus verbundenen LED-Fall wurde Chromosomenuntersuchung vorgenommen. Zahlenmäßige Abweichungen konnten nicht festgestellt werden, bei 18 der 50 untersuchten Zellen ließen sich jedoch konsequent wiederholende Monosomie (Gruppe 12) bzw. Trisomie (Gruppe 22) erkennen. Die Hypothese des Entstehungsmechanismus wird erörtert.

Die vergangenen Jahre brachten auf dem Gebiete der menschlichen Chromosomenuntersuchung bedeutende Ergebnisse. Durch diese zuverläßliche, verhältnismäßig einfache zytogenetische Methodik ist es den Klinikern möglich geworden, eine Anzahl von mit Chromosomenanomalie verbundenen Krankheitsbilder aufzuklären. Obwohl die zwischen Krankheitsbild und Chromosomenabnormalität bestehenden kausalen Zusammenhänge nicht in aller Hinsicht geklärt sind, werden die weiteren systematischen Untersuchungen voraussichtlich zur eingehenden Klärung des Pathomechanismus einzelner Krankheitsgruppen beitragen.

Bisher wurden Chromosomenanomalien von verschiedenen Verfassern bei folgenden Erkrankungen beschrieben: DOWNsche Krankheit [1, 2], KLINEFELTERsches Syndrom [3, 4], TURNERsches-Syndrom [5, 6], »Super-female«-Syndrom [7], HURLERsches-Syndrom [8], MARFANsches-Syndrom [9], STURGE-WEBERsches-Syndrom [10], Leukämie [11, 12], WALDENSTRÖMsche Makroglobulinämie [13, 14], Entwicklungsanomalien [15, 16], Lymphogranulomatose [17, 18], Hämophylie [19], maligne Geschwülste [20], nach Röntgenbestrahlung [21].

Die meisten klinischen Chromsomenuntersuchungen wurden bei verschiedenen intersexuellen Zuständen bzw. bei den Erkrankungen des hämopoetischen Systems durchgeführt. Unsere Zielsetzung war, außer den erwähnten Prozessen auch die sogenannten kollagenen Erkrankungen einer zytologischen Untersuchung zu unterwerfen. Unseres Wissens wurden Chromosomenuntersuchungen bei dieser Krankheitsgruppe nur in Fällen von Rheumatoid-Arthritis vorgenommen. Während CASTOR [22] in aus synovialen Schleimhautzellen verfertigten Kulturen ziemlich hochgradige Aneuplodität beobachten konnten, fand BARTFELD [23] in den Kulturen von peripherem Blut keinerlei Chromosomenabnormalität.

Unseres Erachtens ist die weitere eingehende Untersuchung der Kollagenerkrankungen — trotz dieser, im wesentlichen negativen Ergebnisse — berechtigt und interessant, zumal da diese Krankheiten in erster Reihe bei Frauen vorkommen und häufig auch familiären Charakters sind. Für die Aktualität der Experimente spricht außerdem auch die BURNETsche Theorie (»clonal-selection«) [24], d. h. die Klärung der Frage ob die annehmbar genetisch fremde Zellpopulation, die der »graft versus host«-Reaktion entsprechend die erwähnten Krankheitsbilder verursacht, mit den zur Verfügung stehenden zytogenetischen Methoden diagnostiziert werden kann.

In vorliegender Arbeit wollen wir die anläßlich der Chromosomenuntersuchung eines sich im Bilde von hämolytischem Ikterus manifestierenden Lupus erythematosus-Falles gewonnenen Ergebnisse bekanntgeben.

Falldarstellung

Frau I. K. Die 51jährige Patientin steht seit 1961 unter klinischer Kontrolle. Beim Krankheitsbeginn lagen bei der Patientin Hämoglobinurie und auf hämolytischen Ikterus weisende Symptome vor. Wesentlichere Laboratoriumsbefunde: Blutsenkung nach Westergreen: 66 mm/St. Erythrozytenzahl: 2 000 000, Leukozytenzahl: 3100, Hämatokrit: 12, Serumbilirubin: 1,8 mg%, Diazo: verzögert. Coombs-Test: positiv. Resistenz der Erythrozyten: Hämolyse in sämtlichen Röhrchen. Harn: ausdrückliche Hämoglobinurie. LE-Phänomen: wiederholt positiv. Hauterythem von Lupus-Typ. Steroid- und Chlorochin-Therapie bewirkten auffallend rasche Besserung, und obwohl einigemale noch vorübergehende spärliche Hämoglobinurien vorkamen, ist die Patientin seit einem Jahr praktisch beschwerdenfrei.

Methodik

Die Chromosomenuntersuchungen wurden mit der HUNGERFORDschen Methode [25] durchgeführt. Das durch Sternumpunktion gewonnene Knochenmark wurde in einer Parker 199 und Homologserum enthaltenden Nährlösung suspendiert und in den Thermostat gelegt. Nach 20 Stunden wurde der Kultur 3 γ/ml Colchicin hinzugefügt und noch weitere 2 Stunden hindurch inkubiert. Die mit hypotonischer Lösung behandelten und fixierten Präparate wurden mit Essigsäure-Orcein gefärbt und die geeigneten Chromosomengruppen mit 1000facher Vergrößerung photographiert.

Bewertung

Die ausgeschnittenen und paarweise geordneten Chromosomen wurden dem DENVER-System [26] entsprechend gruppiert. Ausgewertet wurden 50 im Stadium der Metaphase befindlichen Zellen. Bei 18 mitotischen Zellen ließen sich die auf der Abbildung dargestellten, folgerichtig wiederholenden Veränderungen erkennen.

Das Idiogramm veranschaulicht, daß zahlenmäßige Veränderungen nicht vorliegen, in sämtlichen der bewerteten, im Stadium der Metaphase befindlichen Zellen sind 46 Chromosomen zu beobachten. Anläßlich der DENVER-Gruppierung ergibt sich jedoch, daß von den zu Gruppe 22 gehörenden kleinen akrozentrischen Chromosomen anstatt — wie normalerweise — 2, 3 vorzufinden sind und gleichzeitig das eine mittelgroße submediane Chromosom (annehmbar das No. 12) unpaarig ist.

Abb. 1. Idiogramm. No. 22 enthält drei Chromosomen, No. 12 ist unpaarig

Besprechung

Die Entstehung der beschriebenen Chromosomenanomalie dürfte folgendermaßen verlaufen. Bei der Stammzelle der Zellpopulation pathologischen Karyotyps, bei einem der mittelgroßen submedianen Chromosomen erfolgte auf Wirkung irgendeines schädigenden Faktors Fragmentierung in der Nähe des Zentromers. Der das Zentromer enthaltende kürzere Chromosomensatz wurde während der folgenden Teilungen weiter reproduziert, während der andere, kein Zentromer enthaltende Chromosomensatz ausgeschieden wurde. Auf Grund dieser Hypothese kann das laut des DENVER-Systems in Gruppe 22 gehörende, kleine akrozentrische Chromosom als der kürzere Chromosomensatz des fehlenden mittelgroßen submedianen Chromosoms betrachtet werden.

Aus diesem einen Fall wollen wir natürlich keine allgemeinen Schlüsse ziehen. Es ist nicht ausgeschlossen, daß der beobachteten Anomalie lediglich in der Auslösung der Hämolyse eine Rolle zukommt, obwohl bei hämolytischem Ikterus bisher keine Chromosomenanomalien gefunden wurden [27]. Unsere im Gang befindlichen, bei LED-Kranken vorgenommenen Chromosomenuntersuchungen sollen zur Klärung der Frage beitragen, ob es sich hierbei um eine systematisch vorkommende, auf Lupus erythematosus charakteristische Abnormalität, oder um eine nur zufällig vorkommende individuelle Chromosomenanomalie handelt.

LITERATUR

1. LEJEUNE, J., TURPIN, R., GAUTIER, M. L.: Ann. Génét. **1**, 41 (1959).
2. JACOBS, P. A., BAIKIE, A. G., COURT-BROWN, W. M., STRONG, J. A.: Lancet **1**, 710 (1959).
3. JACOBS, P. A., STRONG, J. A.: Nature (Lond.) **183**, 302 (1959).
4. BERGMAN, S., REITALU, J., NOWAKOWSKI, H.: Ann. hum. Genet. **24**, 81 (1960).
5. FORD, C. E., JONES, K. W., POLANI, P. E., DE ALMEIDA, J. C., BRIGGS, J. H.: Lancet **1**, 711 (1959).

6. Fraccaro, M., Kaijsir, K., Lindsten, J.: Lancet **1**, 886 (1959).
7. Jacobs, P. A., Baikie, A. G., Court-Brown, W. M., MacGregor, T. N., MacLean, N.. Harnden, D. G.: Lancet **2**, 423 (1959).
8. Gayler, B. W., Fried, M.: Amer. J. clin. Path. **38**, 590 (1962).
9. Tjio, J. H., Puck, T. T., Robinson, A.: Proc. nat. Acad. Sci. (Wash.) **46**, 532 (1960).
10. Hayward, M. D., Bower, B. D.: Lancet **2**, 844 (1960).
11. Nowel, P. C., Hungerford, D. A.: Science **132**, 1947 (1960).
12. Grouchy, J., Lamy, M.: Rev. franç. **6**, 639 (1962).
13. Benirschike, K., Brownhill, L., Ebough, F. G.: Lancet **1**, 594 (1962).
14. Bottura, C., Ferrari, I., Veiga, A. A.: Lancet **1**, 1170 (1961).
15. Patau, K., Smith, D. E., Therman, E., Inhorn, S. L., Wagner, H. P.: Lancet **1**, 790 (1960).
16. Böök, J. A., Santesson, B.: Lancet **1**, 858 (1960).
17. Ricci, N., Punturieri, E., Bosi, L., Castaldi, G. L.: Lancet **2**, 564 (1962).
18. Spriggs, A. I. Boddington, M. M.: Lancet, **2**, 153 (1962).
19. Elves, M. W., Israels, M. C. G.: Lancet **2**, 909 (1962).
20. Ishihara, T., Moore, G. E., Sandberg, A. A.: Cancer Res. **22**, 375 (1962).
21. Tough, I. M., Buckton, K. E., Baikie, A. G., Court-Brown, W. M.: Lancet **2**, 849 (1960).
22. Castor, C. W.: Arthr. and Rheum. **3**, 436 (1960).
23. Bartfeld, H.: New Engl. J. Med. **267**, 551 (1962).
24. Burnet, F. M.: Clonal Selection Theory of Acquired Immunity. Vanderbilt Univ. Press, Nashville 1959.
25. Hungerford, D. A., Donnelly, A. J., Nowell, P. C., Beck, S. M.: Amer. J. hum. Genet. **11**, 215 (1959).
26. "Denver" Study group: A Proposed Standard System of Nomenclature of Human Mitotic Chromosomes. Lancet **1**, 1063 (1960).
27. Bottura, C., Ferrari, I.: Acta haemat. (Basel) **28**, 20 (1962).

Dr. Sándor Nagy, II. Belklinika, Debrecen 12, Ungarn

THE EFFECTS OF VITAMIN E IN RATS KEPT ON A CARDIOPATHOGENIC DIET

By

GY. SIMON, GY. HARMOS, J. RIGÓ, T. GÁTI, T. KEMÉNY and J. SÓS

INSTITUTE OF PATHOPHYSIOLOGY (DIRECTOR: PROF. J. SÓS)
UNIVERSITY MEDICAL SCHOOL, BUDAPEST

(Received May 29, 1963)

Albino rats — fed on a normal and on a cardiopathogenic diet — were treated with 6 mg of vitamin E. As a result, the decrease of the transaminase (GOT) activity of the heart muscle proved to be slighter in these rats than in those kept on the same diet and not treated with vitamin E. During the experiment, which lasted 30 days, from 18 animals fed the cardiopathogenic diet 9 died with infarctoid cardiopathy, while of the 15 animals kept on the same diet and subjected to vitamin E treatment, only 3 died. In the other examined parameters, no significant differences were detected between the two groups. However, blood pressure, histological findings, phosphatase activity, cholesterol values were nearer to normal in the vitamin E treated animals than in the others. Vitamin E seemed to produce a certain protective effect, but this appeared to be partial and slight.

In recent years, many papers have dealt with the effect of vitamin E in arteriosclerosis as well as in myocardial infarction. As to the results, some authors, for example DOMINGUEZ [1], reported on a favourable protective effect, whereas others [2] observed moderate results. These contradictions might perhaps find their explanation in the different experimental conditions. Our investigations concerning the effect of vitamin E were therefore performed under well-known standard conditions, in animals fed on the S—65 cardiopathogenic diet of Sós. The effect of the diet has been discussed previously [3, 4, 5].

Experimental

In the experiments male albino (Wistar strain) rats, with a starting weight of 100—120 g, were used. The animals were divided into four groups. Seven animals were fed a semi-synthetic normal diet, and six received in addition 6 mg of vitamin E (0.2 ml vitamin E, Richter, Budapest). The third group containing 18 animals, was kept on the S-65 cardiopathogenic diet. The fourth group (15 animals) received the same cardiopathogenic diet, and subcutaneously 6 mg of vitamin E in oil. The diet of all the animals amounted to 10 g daily. Water was not restricted. During four weeks body weight and blood pressure were measured regularly. The time of accidental death was registered. On the 29th day of the treatment the animals were sacrificed by decapitation.

Total cholesterol as well as esterificated cholesterol in serum were determined by the Lieberman reaction. The hearts were studied histologically, and after homogenization examined for acid and alkaline phosphatase by Bodansky's method as modified by PRATT [6], and for glutamic-oxaloacetic transaminase (GOT) activity by the modified method of DUBACH [7]. From the aorta samples weighing about 10 mg were taken, and dissolved in sulphuric acid diluted 1 : 1, in the presence of H_2O and $CuSO_4$. In further dilutions the Ca content was determined by flame photometry.

Results

The results are summarized in the following table.

Table

	Normal	Normal + Vit. E.	S-65	S-65 + Vit. E.
Number of animals at the beginning	7	6	18	15
Number of died animals	0	0	9	3
Number of animals subjected to chemical investigations	7	6	9	12
Changes in body weight, per cent	+ 25	+ 38	— 5	— 4
Blood pressure maximum, mm Hg	123 ± 6	127 ± 8	166 ± 17	154 ± 4
Total cholesterol in serum, mg per 100 ml	129 ± 39	89 ± 29	281 ± 80	246 ± 84
Cholesterol ester in serum in percentage of total cholesterol	43 ± 17	41 ± 15	76 ± 18	56 ± 19
Acid phosphatase content of heart muscle μg P/g heart muscle/hour	67 ± 16	59 ± 13	53 ± 19	61 ± 22
Alkaline phosphatase content of heart muscle μgP/g heart muscle/hour	267 ± 30	323 ± 58	275 ± 64	294 ± 69
Glutamic-oxaloacetic transaminase content of heart muscle mg pyruvic acid/mg heart muscle/hour	408 ± 19	381 ± 39	316 ± 32	361 ± 47
Ca content of aorta, per cent	0.58 ± 0.16	0.61 ± 0.15	0.69 ± 0.19	0.67 ± 0.14

According to the statistical evaluation with Student's t test, the difference between the S-65 and S-65 + E vitamin group was significant concerning esterificated cholesterol ($p<0.02$) and GOT activity ($p<0.05$). Regarding the other data of these groups, the difference was not significant.

Histology of the aorta showed no difference between the two groups. There was a characteristic unevenness of the elastic fibres, with occasional elastolysis. In the S-65 group the heart of one animal out of the nine was histologically intact (none of the animals which died after the 20th day had an intact heart), whereas in the S-65+ vitamin E group the heart of four out of 12 was intact. The changes were as described previously [3]; as to the gravity of the lesions, there was hardly any difference between the two groups.

Discussion

The main cardiopathogenic factors of the S-65 diet are its richness in Na, Cl, and Ca, poverty in K and Mg, large amounts of vitamin D, cholic acid salts, cholesterol, perchlorate. Under the conditions applied, this diet produced myocardiac necroses in about 30 days. In the animals dead spontaneously

between the 20th and 29th days of the experiment, similar changes could be observed. The low number of dead animals in the vitamin E-treated group was striking and perhaps not accidental, although owing to the small number of animals no conclusions could be drawn.

The animals treated with vitamin E were somewhat nearer to normal (with respect to blood pressure, serum total cholesterol, aortic calcium, myocardial histology) than the group which was offered only the S-65 diet. The differences were, however, slight and not significant statistically.

The protective effect of vitamine E manifested itself in the GOT activity of the homogenized heart muscle, whereas in the group kept on the S-65 diet, the most damaged animals, showing at the same time the lowest GOT activity, could not be examined as they died before time.

The increase of the ratio serum cholesterol ester per total cholesterol is a striking effect of the S-65 diet, the mechanism of which is unknown. It might perhaps be explained with the diminished reticuloendothelial function or a disturbance of the lymph circulation. Under the effect of vitamin E the ratio was significantly decreased. The vitamin E content of the cardiopathogenic diet S-65 is somewhat lower than the normal requirement, but this presumably was not a pathogenic factor in an experiment lasting 29 days, it being known that in rats several months are needed for symptoms to arise on a vitamin E deficient diet. However, the perchlorate and iron content of the S-65 diet probably enhances the utilization of vitamin E.

Our experiments have failed to furnish a definite answer to the question whether large doses of vitamin E had a protective effect in case of cardiopathogenic diet. Our results seem to suggest that a certain protective effect is produced; this, however, is only partial, and presumably slight.

REFERENCES

1. Puente-Dominguez, J. L., Dominguez, R.: Rev. esp. Cardiol. **9**, 30 (1955).
2. Harman, D.: Circulation **22**, 151 (1960).
3. Sós, J., Gáti, T., Kemény, T., Rigó, J., Budavári, I.: Acta med. Acad. Sci. hung. **16**, 189 (1960).
4. Rigó, J., Budavári, I., Sós, J.: Acta med. Acad. Sci. hung. **17**, 85 (1961).
5. Sós, J.: Magy. Tud. Akad., Biol. orv. Tud. Osztály Közl. **12**, 91 (1961).
6. Pratt, O.: Biochem. J. **55**, 140 (1953).
7. Dubach, A. C.: Schweiz. med. Wschr. **87**, 185 (1957).

Dr. György Simon,
Dr. György Harmos
Dr. János Rigó
Dr. Tibor Gáti
Dr. Tibor Kemény
Dr. József Sós

Budapest IX., Hőgyes E. u. 9.

EFFECTS OF HYALURONIDASE AND ANTIDIURETIC HORMONE ON FLOW AND COMPOSITION OF THE RENAL LYMPH

By

M. PAPP and KATALIN SZALAY

DEPARTMENT OF PATHOPHYSIOLOGY, RESEARCH INSTITUTE OF EXPERIMENTAL MEDICINE (DIRECTOR: PROF. I. RUSZNYÁK), HUNGARIAN ACADEMY OF SCIENCES, BUDAPEST

(Received May 31, 1963)

Hyaluronidase in a dose of 600 V. R. injected into the renal artery brought about a significant fall in diuresis and lymph protein concentration, and ADH a significant rise in lymph K^+ content. In control animals treated similarly with physiological saline no such effects were observed.

In renal physiology a wealth of knowledge has been acquired, yet we know but little of the relationship between renal function and renal lymph circulation. Still, it may be rewarding to study this relationship on at least two accounts. First, it is still somewhat difficult to interpret the physiological significance of the renal lymphatics. Secondly, investigations into the composition of the lymph may allow some insight into the dynamics of the renal interstices. The part played by the antidiuretic hormone (ADH) in the regulation of urinary osmotic concentration is common knowledge. GINETZINSKY [1] observed that after ADH administration structures reminiscent of lymph capillaries appeared in the region of the collecting tubules. They assigned to these structures a role in the transport of water which upon the action of ADH enters the interstitial space from the collecting tubules. In their opinion, which has been questioned in the literature, it is by releasing hyaluronidase that ADH renders permeable to water the membrane separating the tubule lumen from the interstitial tissue. The recent electron microscopic studies by AMON and GAYER [2] have shown hyaluronidase to be capable of causing structural changes in the glomerular membrane.

In the experiments to be discussed renal lymph flow and composition were studied under the effect of hyaluronidase and ADH, substances which under adequate circumstances are causing a concentration of urine.

Methods

Dogs of either sex, weighing between 12 and 20 kg, were anaesthetized with 0.03 g/kg body weight of pentobarbital. Urine was collected through a polyethylene cannula bound into the left ureter, and lymph through a cannula introduced into a hilar lymphatic of the left kidney. All the other visible lymph vessels were ligated. One group of the experimental animals was infused intravenously with 5 per cent dextrose, to maintain adequate diuresis. Following an initial period of urine and lymph collection, 600 V. R. of hyaluronidase (Hyason,

Organon) or 5 to 10 I. U. of vasopressin (Tonephin, Hoechst or Piton, Organon) were injected into the left renal artery, depending on the type of the experiment. Control animals received adequate amounts of physiological saline. After the above treatment another period of lymph and urine collection was started. The collection periods varied in duration between 45 and 90 minutes, depending upon the rate of flow. Blood pressure was measured in the femoral artery. To prevent blood clotting, the animals were given intravenously 0.05 ml/kg of heparin, and powdered heparin was added to the blood and lymph samples. Total protein was determined with the biuret method, serum and protein fractions by electrophoresis, Na^+ and K^+ concentrations by flame photometry, and osmotic concentration by freezing point depression using Beckmann's thermometer.

Results and discussion

I. The hyaluronidase effect was studied in 9 dextrose-hydrated and 4 normal dogs. Fig. 1 shows that 600 V. R. of the enzyme reduced the average lymph protein content from 2.93 g to 2.26 g per 100 ml ($p<5\%$) and minute diuresis from 0.44 ml to 0.18 ml ($p<1\%$); it further shows that the A/G quotient was

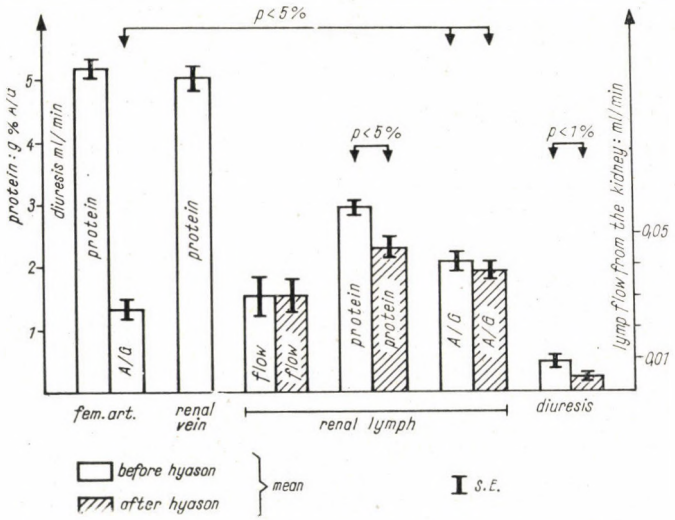


Fig. 1. Effect of hyaluronidase on the flow and protein content of renal lymph (13 animals)

throughout higher for the lymph than for the plasma, being 2.0 and 1.3, respectively ($p<5\%$). As illustrated in Fig. 2, the hyaluronidase had no appreciable effect on the Na^+ and K^+ contents and the osmotic concentration of the lymph. It is necessary to mention here that in this experimental group the Na^+ content during the initial period was significantly higher in the lymph than in the blood plasma ($p<1\%$).

The outcome of these experiments indicate that lymph flow, osmotic concentration and the A/G quotient of lymph remained unchanged after hyaluronidase administration. Surprisingly, the enzyme — and perhaps its administration itself — was found to have reduced the lymph protein content. On the other hand, a comparison of the decrease in lymph protein concen-

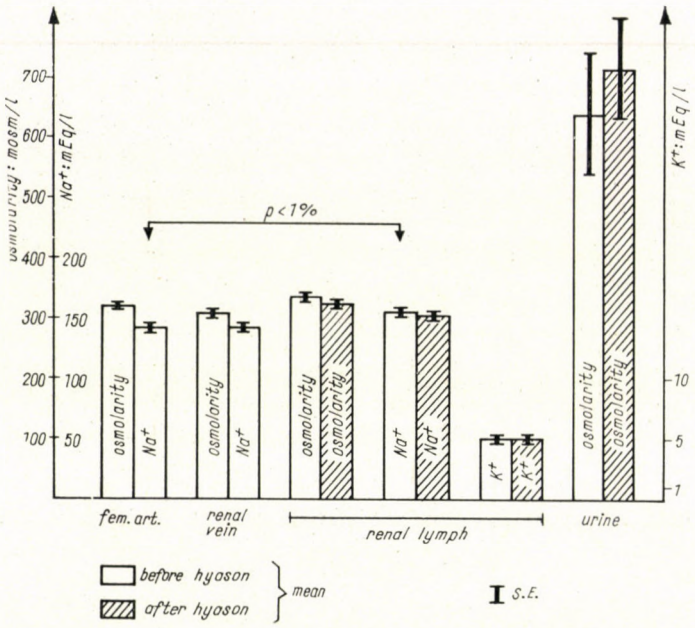


Fig. 2. Effect of hyaluronidase on the osmolarity of renal lymph and urine (13 animals)

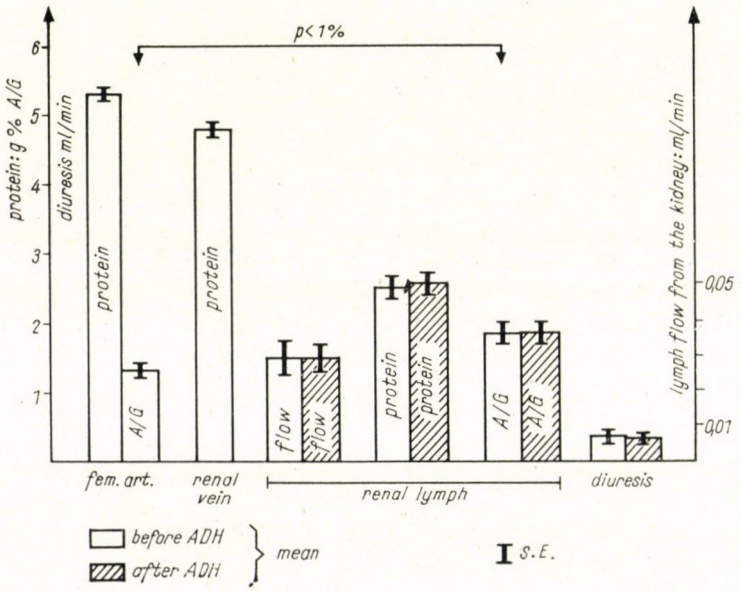


Fig. 3. Effect of ADH on the flow and protein content of renal lymph (20 animals)

tration after hyaluronidase with that after physiological saline revealed no significant difference between the two groups ($p>20\%$). Accordingly Hyason may act differently on capillary permeability in the kidney, than in other

regions of the organism, where on the evidence of data in the literature [3, 4, 5], it increases capillary permeability, enhances the lymph flow in the thoracic duct, the liver, the neck, and the gastrointestinal tract [4]; it also raises the globulin content of the lymph, without influencing the total protein [4, 5].

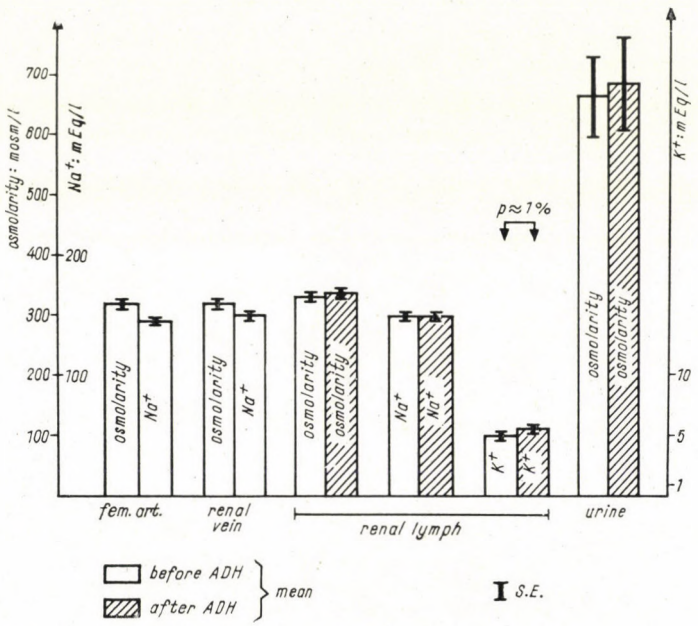


Fig. 4. Effect of ADH on the osmolarity of renal lymph and urine (20 animals)

According to AMON and GAYER [2], chronic hyaluronidase administration increases the permeability of the basement membrane. In our acute experiments, however, hyaluronidase did not significantly influence the permeability of renal blood capillaries, perhaps owing to the enzyme's rapid decomposition by the kidney. The cause of the decrease in the protein content of renal lymph is unknown.

II. ADH was studied for its effect upon renal lymph composition in 6 dextrose-hydrated and 14 normal dogs. Fig. 3 shows that ADH did not significantly decrease either lymph flow or diuresis. In the initial period the A/G quotient was significantly higher in the lymph (1.86) than in the plasma (1.32) ($p<1\%$). Fig. 4 illustrates the significant increase in the lymph K^+ content from 5.06 mEq to 5.42 mEq ($p\approx1\%$), upon the action of ADH, which, however caused no change in the plasma K^+ level.

In the dextrose-hydrated animals ADH reduced minute diuresis from 0.71 ml to 0.53 ml, while the average rate was left unchanged in the non-hydrated animals. In the dextrose-hydrated animals the Na^+ concentration during

the initial collection period was higher in the lymph (146.5 mEq) than in the plasma (142.2 mEq); the difference was significant statistically ($p<5\%$).

Physiological saline injected into the renal artery of the 6 control animals failed to change the lymph's protein content and K^+ concentration (Figs 5, 6).

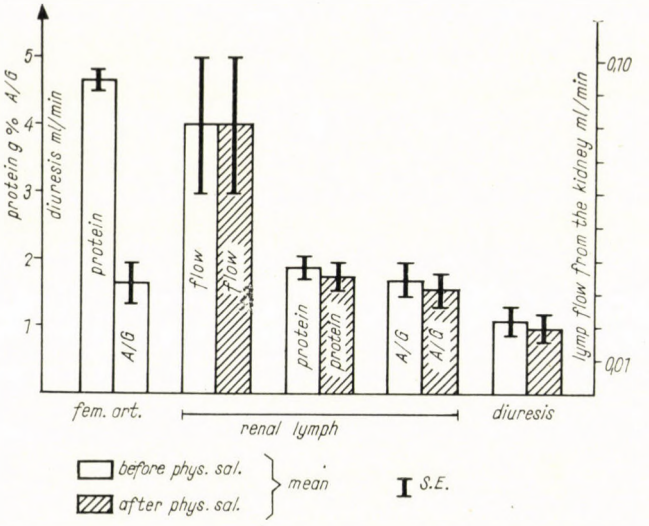


Fig. 5. Effect of physiological saline on the flow and protein content of renal lymph (6 animals)

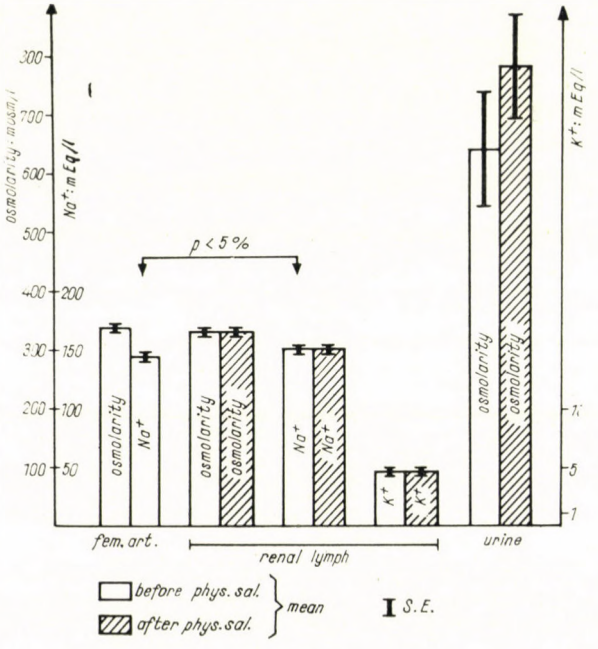


Fig. 6. Effect of physiological saline on the osmolarity of renal lymph and urine (6 animals)

In conclusion, ADH produced no change in either the flow or the osmotic concentration of lymph, independently of whether diuresis decreased or remained unchanged. On the other hand, it increased the lymph's K^+ level, regardless of the hormone's effect on the rate of diuresis.

The effect of ADH to enhance urinary K^+ excretion has been described earlier by SARTORIUS et al [6], BERLINER [7], BERLINER et al. [8], and BROOKS et al. [9], ANSLOW et al [11]. The effect depends on the K^+ content of the cells of the distal tubule (BERLINER, 7) and the degree of cellular hydration (MUDGE et al., 10). Our findings appear to show that under the effect of ADH there is an increased loss of K^+ not only towards the tubular lumen but also into the interstitial space of the kidney.

REFERENCES

1. GINETZINSKY, A. G.: Role of hyaluronidase in the re-absorption of water in renal tubules: the mechanism of action of the antidiuretic hormone. Nature (Lond.) **182,** 1218 (1958).
2. AMON, H., GAYER, I.: Elektronenmikroskopische Untersuchungen über den Einfluß der Hyaluronidase auf die Basalmembran der Glomerulumcapillaren mit besonderer Berücksichtigung der Permeabilitätsfrage. Klin. Wschr. **41,** 163 (1963).
3. ELSTER, S. E., FREEMAN, M. E., ANDERSON, P. R.: The effect of hyaluronidase on the haematocrit and plasma proteins in the albino rat. J. lab. clin. Med. **34,** 834 (1949).
4. SZABÓ, G., MAGYAR, S.: Die Wirkung von Hyaluronidase auf die Capillarpermeabilität und Lymphbildung. Z. ges. exp. Med. **130,** 129 (1958).
5. FÖLDI, M., PAPP, N., KISFALUDY, A., STEKKER, K.: Die Wirkung von Hyaluronidase auf den Transport verschiedener Plasmaproteine durch den Ductus thoracicus. Clin. chim. Acta **5,** 839 (1960).
6. SARTORIUS, O. W., ROBERTS, K.: The effects of pitressin and desoxycorticosterone in low dosage on the excretion of sodium, potassium and water in the dog. Endocrinology **45,** 273 (1949).
7. BERLINER, R. W.: Renal excretion of water, sodium, chloride, potassium, calcium and magnesium. Amer. J. Med. **9,** 541 (1950).
8. BERLINER, R. W., KENNEDY, TH. J. JR., HILTON, J. G.: Renal mechanismus for excretion of potassium. Amer. J. Physiol. **162,** 348 (1950).
9. BROOKS, F. P., PICKFORD, M.: The effect of posterior pituitary hormones on the excretion of electrolytes, in dogs. J. Physiol. (Lond.) **142,** 468 (1958).
10. MUDGE, G. H., FOULKS, J., GILMAN, A.: Renal secretion of potassium in the dog during cellular dehydratation. Amer. J. Physiol. **161,** 159 (1950).
11. ANSLOW, P., JR., WESSON, L. G.: Some effects of pressor-antidiuretic and oxytocic fractions of posterior pituitary extract on sodium, chloride, potassium and ammonium excretion in the dog. Amer. J. Physiol. **182,** 561 (1955).

Dr. Miklós PAPP
Dr. Katalin SZALAY } Budapest VIII., Korányi S. u. 2, Hungary

THE EFFECT OF BLOOD CORTICOID LEVELS ON ACTH-RELEASE CAUSED BY STRESS

By

E. STARK and J. FACHET

DEPARTMENT OF PATHOPHYSIOLOGY, RESEARCH INSTITUTE OF EXPERIMENTAL MEDICINE (DIRECTOR: I. RUSZNYÁK), HUNGARIAN ACADEMY OF SCIENCES, BUDAPEST, HUNGARY

(Received August 22, 1963)

The question has been studied whether or not corticosterone secretion caused by non-specific stress is suppressed by a high blood corticoid level in the rat.

It was found that whereas pharmacological doses of corticoids suppressed the release of ACTH in the non-stressed animals, they did not inhibit ACTH-release due to formalin: this in spite of the fact that the exogenous hormon levels considerably exceeded the corticosterone concentration following stress.

It would appear that the mechanism of the negative feed-back cannot be invoked as the sole explanation for the ACTH-release due to stress.

In earlier studies of the mechanism of the stage of resistance elicited by non-specific stimuli, it was shown that 24 hours after the last injection of prolonged ACTH treatment, formalin as a non-specific stress produced no rise in the corticosterone level of the peripheral blood, although this level was normal or below normal, and the adrenals responded with increased hormone production to ACTH both in vivo and in vitro [1, 2].

The suggestion of SAYERS and SAYERS [3, 4] that the secretion of ACTH is regulated by changes in the blood corticoid level is now widely adopted but not unchallenged, though a number of authors had presented experimental evidence that pretreatment with corticoids inhibited stress-induced ascorbic acid depletion [5, 6, 7, 8, 9, 10] and elevation of the corticosterone level of the peripheral blood [11, 12].

Our own findings referred to above [1, 2], and some observations of other workers [13, 14, 15] which it is not possible to interpret by the negative feed-back mechanism, prompted us to undertake direct corticoid measurements to see if in the presence of a high peripheral hor mone level, produced by exogenous corticoids, stress would elicit increased adrenal activity.

Material and methods

Wistar-strain rats of both sexes, maintained on a standard diet and weighing between 180 and 220 g, were fasted for 24 hours before the beginning of the experiments, with water available ad lib.

The stressful stimulus was a single subcutaneous injection of 0.5 ml of 3% formalin. Control animals were given physiological saline in the same volume. The animals were decapi-

Table I

Changes in the corticosterone level of the peripheral blood after formalin in female rats pretreated with hydrocortisone and prednisolone

Experimental variables in pretreatment	In hours preceding stress	At time of application of stress		One hour after application of stress corticosterone μg/100 ml
		exogenous corticoid μg/100 ml	corticosterone μg/100 ml	
0.5 ml of phys. saline s.c.	1	—	20.9 ± 3.9 (6)*	93.8 ± 5.0 (11)
0.5 ml of phys. saline s.c.	4	—	24.6 ± 4.7 (6)	89.5 ± 5.8 (6)
20 mg/100 g hydrocortisone s.c.	15	—	11.8 ± 4.1 (6)	82.3 ± 3.4 (6)
10 mg/100 g hydrocortisone s.c.	4	—	17.3 ± 3.1 (6)	92.0 ± 7.3 (6)
6 mg/100 g hydrocortisone i.p.	4	29.7 ± 1.8 (6)	5.9 ± 0.8 (6)	50.0 ± 6.8 (6)
6 mg/100 g hydrocortisone i.p.	1	545.7 ± 40.0 (6)	0.8 ± 0.4 (11)	48.1 ± 3.4 (11)
8 mg/100 g prednisolone i.p.	1	486.3 ± 37.9 (6)	2.7 ± 0.7 (6)	70.2 ± 3.8 (6)

* number of determinations

Table II

Changes in the corticosterone level of the peripheral blood after formalin in male rats pretreated with hydrocortisone

Corticosterone μg/100 ml				Hydrocortisone μg/100 ml	
at time of application of stress		one hour after application of stress			
in saline treated rats	in hydrocortisone treated rats	in saline treated rats	in hydrocortisone treated rats	at time of application of stress*	one hour after application of stress
10.0 ± 1.1 (11)**	1.0 ± 0.5 (10)	31.8 ± 2.7 (11)	13.7 ± 1.7 (11)	174.3 ± 21.9 (9)	72.0 ± 9.8 (11)

* one hour after i.p. injection of 6 mg/100 g body weight
** number of determinations

tated one hour after the application of the stress. Preceding it, hydrocortisone,* prednisolone** and physiological saline, respectively, was given in doses, and under conditions, as detailed in Tables 1 and 2.

To arrive at the actual concentrations of the exogenous corticoids in the peripheral blood at the time the stress was applied, concentration determinations were made in additional groups of rats 1 and 4 hours after the administration of the corticoids. Corticosterone and exogenous corticoid levels in the blood were always measured simultaneously one hour after formalin y paper chromatography, using the method described in earlier work [2].

Results and comments

Table I summarizes the results obtained in the female rats. The applied doses of corticoids (with the exception of the 10 mg dose of hydrocortisone s. c.) significantly reduced the corticosterone level of the peripheral blood in relation to the controls. The high concentration in the blood of hydrocortisone or prednisolone failed to inhibit the formalin-induced rise in the corticosterone level in the non-stressed animals, but it was less than in the non-pretreated animals.

Table II presents the slightly different results obtained in the male rats. Like in the female rats, hydrocortisone substantially reduced the blood corticosterone level in the non-stressed animals, and although the rise produced by formalin in the corticosterone concentration of the animals was from 1.0 mg to 13.7 mg/100 ml ($p<0.01$), it remained below that caused by formalin in animals not pretreated with corticoids.

Our results appear to show that pharmacological doses of exogenous corticoids suppress the release of ACTH in the control animals, but do not inhibit ACTH mobilization caused by non-specific stress.

Our findings and the probable causes of differences from those of YATES et al. will be discussed in detail in a future publication.

Acknowledgement

The authors wish to thank Mr. Béla Hajtman for preparing the statistical analyses.

* * *

Note added in proof. Recent experimental data of HODGES and JONES (J. Physiol. **167,** 30, (1963) and SMELIK (Acta endocr. (Kbh.) **44,** 36 (1963)) are in certain respects in agreement with our findings.

REFERENCES

1. FACHET, J., STARK, E., MIHÁLY, K.: Acta Physiol. Hung. (Suppl.) **20,** 59 (1962).
2. STARK, E., FACHET, J., MIHÁLY, K.: Canad. J. of Biochem. and Physiol. **41,** 1771 (1963).
3. SAYERS, G., SAYERS, M. A.: Endocrinology **40,** 265 (1957).
4. SAYERS, G., SAYERS, M. A.: Ann. of N. Y. Acad. Sci. **50,** 522 (1950).
5. PORTER, J. C., RUMSFELD, H. W.: Endocrinology **58,** 359 (1956).
6. PORTER, J. C., JONES, J. C.: Endocrinology **58,** 62 (1956).
7. MCCANN, S. M.: Endocrinology **60,** 664 (1957).

* Hydrocortisone-acetat microcryst. susp.
** Di-adreson-F aquosum Organon OSS.

8. HODGES, J. R., VERNIKOS, J.: Brit. J. Pharmacol **13,** 98 (1958).
9. DE WIED, D., MIRSKY, J. A.: Endocrinology **64,** 955 (1959).
10. BARRETT, A. M.: Brit. J. of Pharmacy and Pharmacol. **13,** 20 (1961).
11. YATES, F. E., LEEMAN, S. E., GLENISTER, D. W., DALLMAN, M. F.: Endocrinology **69** 67 (1961).
12. LEEMAN, S. E., GLENISTER, D. W., YATES, F. E.: Endocrinology **70,** 249 (1962).
13. FORTIER, C., YRARRAZAVAL, S., SELYE, H.: Am. J. Physiol. **165,** 466 (1951).
14. COX, G. S., HODGES, J. E., VERNIKOS, J.: J. Endocrin. **17,** 177 (1958).
15. FORTIER, C., DE GROOT, J.: Am. J. Physiol. **196,** 589 (1959).

DR. Ervin STARK
DR. József FACHET } Budapest VIII., Szigony u. 43. Hungary.

Printed in Hungary

A kiadásért felel az Akadémiai Kiadó igazgatója Műszaki szerkesztő: Farkas Sándor
A kézirat nyomdába érkezett: 1963. XI. 18. — Terjedelem: 7,50 (A/5) ív, 24 ábra

64.58033 — Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György

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ТОМ XIX — ВЫП. 4

РЕЗЮМЕ

ДЕЙСТВИЕ НОРАДРЕНАЛИНА НА КРОВООБРАЩЕНИЕ В ГОЛОВНОМ МОЗГУ И НА ОБМЕН ВЕЩЕСТВ ПРИ ИШЕМИЧЕСКОМ ШОКЕ

Д. САБО

У собак при экспериментальном ишемическом шоке нормализация кровяного давления вливанием 1-норадреналина не изменяет выживаемость животных. При повышении кровяного давления ускоряется также кровообращение в головном мозгу и расход кислорода, однако, норадреналин повышает сосудистое сопротивление в головном мозгу, и поэтому между повышением кровяного давления и ускорением кровообращения нет линейной связи.

ИССЛЕДОВАНИЕ АДАПТИВНОГО СИНТЕЗА ЭНЗИМОВ

Ш. ДАН, Л. АНТАЛ, ДЬ. СЕГЕДИ и А. ДЬЁРФФИ

У крыс, отравленных четыреххлористым углеродом снижается содержание триптофанпирролазы в печени и нарушается ее адаптивный синтез.

Для бескровного исследования энзимной адаптации авторы на крысах разработали способ двойной нагрузки триптофаном. При действии индуцирующей первой дозы триптофана повышается содержание триптофанпирролазы в печени. После дачи второй дозы триптофана производные кинуренина и антраниловой кислоты синтезируются в большом количестве и выделяются с мочой.

Исследования двойной нагрузки триптофаном, комбинированные с гистидином и кортизоном, также подтверждают вышесказанное.

ИССЛЕДОВАНИЕ СТЕРОИДОВ МОЧИ ПРИ ГИРСУТИЗМЕ И ВИРИЛИЗМЕ

М. ЮЛЕС, И. ФАРЕДИН, И. ТОТ, А. ДАВИД, К, КОВАЧ

Авторы на основании патологической картины и прочих диагностических (рентгеновское, гинекологическое и т. д. обследования) исследований распределили 49 больных страдавших гирсутизмом, на четыре группы и исследовали у них выделение с мочой нейтральных общих — 17 — стероидов, 17-кетостероидных фракций (β-17-кетостероидов, андростерона, этиохоланолона, 11-оксигенизированных-17-кетостероидов) фракций общих 17-гидроксикортикостероидов, прегнандиола и эстрогена (эстрон, эстрадиол-17-β, эстроол).

Согласно полученным результатам, при помощи этих методов исследования можно дифференцировать только больных с гирсутизмом, исходяшим из коры надпочечников, от больных с гирсутизмом прочего происхождения, и эти лабораторные исследования не позволяют дальнейшее, более точное дифференцирование. Авторы установили, далее, что при гирсутизме выделяется патологически повышенное количество этиохоланолона и выделение эстрона и эстрадиола уменьшается. В статье на основании новейших литературных данных обсуждаются причины патологического выделения этих стероидов. В связи с патогенезом гирсутизма выдвигается вопрос о значении измененной реактивности периферических тканей, и клеток.

СРАВНИТЕЛЬНОЕ КЛИНИЧЕСКОЕ ИЗМЕРЕНИЕ ДЕЙСТВИЯ ПРОТИВОСУДОРОЖНЫХ СРЕДСТВ

(Данные к клинической фармакологии Но—Спа)

Г. ПЕТРАНЬИ и Г. СЕГЕДИ

1. Обычное внутриартериальное сосудорасширяющее лечение болезней, сопровождающихся сужением артерий нижних конечностей, применимо для измерения действия противоспазматических средств, действующих на гладкие мышцы, и его можно рекомендовать в качестве клинико-фармакологического теста.

2. При помощи этого теста проводилось сравнительное исследование перпарина, папаверина и нового производного, дигидроперпарина.

3. Дигидроперпарин (Но—Спа, Хиноин,) оказался в два раза более эффективным других указанных средств, причем он в достаточно эффективных дозах не имеет побочных действий, и следовательно предоставляет возможность для сбережения наркотических средств.

СОДЕРЖАНИЕ ГИСТАМИНА В ПЕРЕСАЖИВАЕМОЙ ТУЧНОКЛЕТОЧНОЙ ОПУХОЛИ

П. ГРОФ и Г. КЕЛЕНЬИ

Авторы пытались произвести пересадку перевиваемой мышечной мастоцитомы *Фурта* на мышечный штамм собственного разведения. Морфологические свойства вызванных опухолей исследовались под световым и электронным микроскопами. Авторы проверяли образование метастазов гистологическим методом исследования и определили биологическим методом содержание гистамина в опухолях и в органах опухолевых мышей. Полученные результаты следующие:

1. Трансплантация мастоцитомы *Фурта* у взрослых здоровых мышей собственного разведения в 10—15% привела к образованию опухоли; у предварительно облученных рентгеновыми лучами животных опухоли возникли в 20—24% случаев.

2. Содержание гистамина в мастоцитомах (8 случаев) было очень высокое (120,0—1114,0 μг/г). В молодых, менее развитых опухолях (ниже 1 г) концентрация гистаминабыла в общем выше, чем в более развитых старших опухолях. В коже окружности опухоли содержание гистамина также было повышено (спаяки) по сравнению с отдаленной от опухоли кожей.

3. В *органах* опухолевых мышей содержание гистамина не было повышено ни в одном случае, что говорит против наличия метастазов и соответствует результатам гистологических исследований.

4. В противоположность этому содержание гистамина всех исследованных органов мышей LAF_1 было очень высокое (в соответствии с этим с гистологически доказанными тучноклеточными инфильтратами), в частности в печени: 1600 μг/г.

5. Авторы дают одинаковое объяснение сравнительно более высокому содержанию гистамина в молодых опухолях мышей собственного разведения и в печени мышей LAF_1: в обоих случаях более благоприятны условия для повышенного образования гистамина.

ДЕЙСТВИЕ НОРАДРЕНАЛИНА НА КРОВОСНАБЖЕНИЕ И РАБОТУ СЕРДЦА ПРИ ИШЕМИЧЕСКОМ ШОКЕ

Д. САБО и Ж. МАДЬЯР

У собак при ишемическом шоке минутный объем, в сущности, снижается параллельно кровяному давлению. Коронарная циркуляция также замедляется, хотя в несколько меньшей мере. Коронарное сопротивление не показывает достоверных изменений.

После вливания норадреналина артериальное давление нормализовалось, однако, минутный объем не повысился достоверно. Причиной повышения кровяного давления было усиление периферического сопротивления. Коронарное же сопротивление не повысилось, а скорее снизилось. В соответствии с этим коронарная циркуляция повысилась выше контрольных величин и коронарная фракция минутного объема также сильно возросла.

АНОМАЛИЯ ХРОМОСОМ ПРИ ГЕМОЛИТИЧЕСКОЙ ЖЕЛТУХЕ, СОПРОВОЖДАЮЩЕЙ РАССЕЯННУЮ КРАСНУЮ ВОЛЧАНКУ

Ш. НАДЬ

Сообщаются результаты исследования хромосом больного, страдающего гемолитической желтухой, сопровождающей рассеянную красную волчанку. Разница в числе хромосом не обнаружена, однако, у 18 из 50 исследованных клеток наблюдалась последовательно повторяющаяся 12-ая моносомия или 22-ая трисомия. Излагается предположительный механизм возникновения этой аномалии хромосом.

ДЕЙСТВИЕ ВИТАМИНА Е НА КРЫС, СОДЕРЖАВШИХСЯ НА КАРДИОПАТОГЕННОЙ ДИЕТЕ

Д. ШИМОН, Г. ХАРМОШ, Й. РИГО, Т. ГАТИ, Т. КЕМЕНЬ и Й. ШОШ

Авторы давали большие дозы (6 мг в день) витамина Е крысам-альбиносам, содержавшимся при нормальной и при кардиопатогенной диетах. Под влиянием дачи витамина Е снижение активности трансаминазы сердечной мышцы было меньше чем у другой группы, получившей ту же самую диету. В течение 30 дней опыта 9 из 18 животных, содержавшихся при кардиопатогенной диете, погибли, вследствие инфарктоидной кардиопатии, в то время как из 15 животных, получивших кроме той же самой диеты также витамин Е погибло только 3. Между другими исследованными параметрами подопытных двух групп авторам не удалось выявить значительной разницы, хотя величины животных, получивших также витамин Е во всех случаях более подходили к норме(кровяное давление, гистологические данные, фосфатазная активность, холестерол).

Авторы придерживаются того мнения, что витамин Е при примененных ими экспериментальных обстоятельствах оказал определенное защитное действие, хотя это действие было только частичное и сравнительно незначительное.

ДЕЙСТВИЕ ГИАЛУРОНИДАЗЫ И АНТИДИУРЕТИЧЕСКОГО ГОРМОНА НА ПОЧЕЧНОЕ ЛИМФООБРАЩЕНИЕ И ЛИМФООБРАЗОВАНИЕ

М. ПАПП и К. САЛАИ

Впрыскивание 600 VRE (единицу понижени вязкости) гиазона в почечную артерию вызвало значительное снижение диуреза и концентрации протеина в лимфе, а впрыскивание АДГ обуславливало значительное повышение содержания K^+ в лимфе. У контрольных животных, получивших физиологический раствор соли подобных эффектов не наблюдалось.

ДЕЙСТВИЕ КОНЦЕНТРАЦИИ КОРТИКОСТЕРОНОВ В КРОВИ НА МОБИЛИЗАЦИЮ АКТГ, ВЫЗВАННУЮ СТРЕССОМ

Е. ШТАРК и Й. ФАХЕТ

Авторы экспериментально изучали вопрос о том, препятствует ли у крыс высокий уровень кортикоидов крови мобилизации АКТГ, вызванной стрессом.

Установлено, что кортикоиды при даче фармакологических доз у животных без стресса почти полностью задерживают выделение АКТГ, но они не препятствуют его мобилизации в состоянии стресса.

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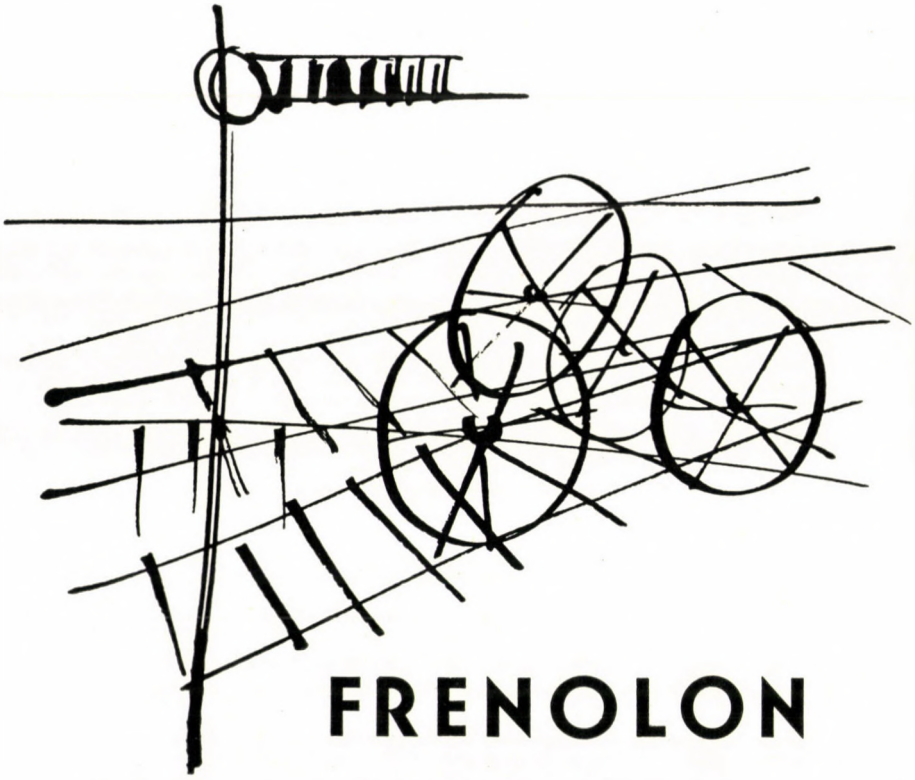

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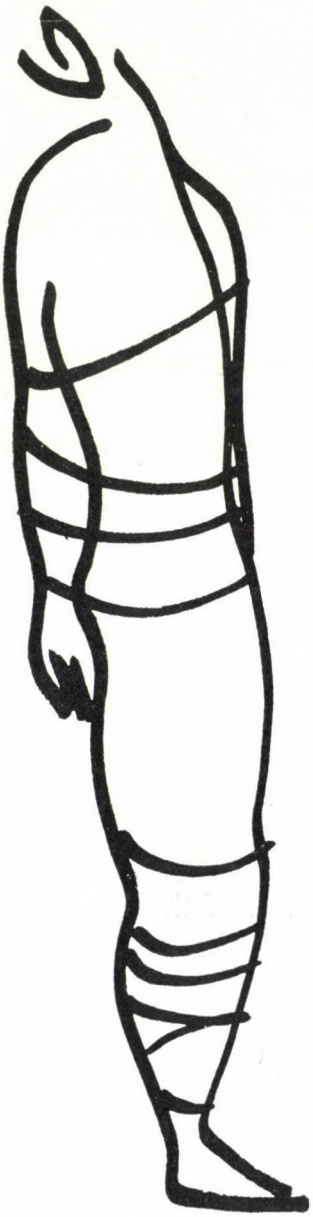

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