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GRANULOCYTE ALKALINE PHOSPHATASE ACTIVITY IN HODGKIN'S DISEASE

By

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(Received March 12, 1970)

Granulocytic alkaline phosphatase activity has been studied in 20 subjects with Hodgkin's disease. Any deterioration of the clinical condition was associated with an early increase of GAP activity which, in the context of the entire clinical picture and of other laboratory evidence, may assist in the early assessment of clinical deterioration or recurrences of the disease.

WACHSTEIN [22] in 1946 was the first to draw attention to the clinical significance of granulocytic alkaline phosphatase (GAP). This phospho-monoesterase which requires an alkaline medium for its activity, is an intracellular enzyme. Its presence is demonstrable semi-quantitatively, by histochemical procedures making use of various azo-dyes which have the capacity of forming deposits of water-insoluble precipitates at the sites of hydrolysis. The extent of the deposits thus formed being related to the activity of GAP serves as its indicator accessible to direct reading. GAP is assumed to be involved in the glycogen synthesis of leukocytes, the increase of which goes parallel with an enhanced enzymatic activity [6, 7, 23]. It also seems to be connected with various hormonal and genetic factors [1, 4, 19, 20]. An increased GAP activity was furthermore noted in connection with pyogenic infections, surgical interventions, obstructive jaundice, pregnancy and steroid therapy [5, 9, 17, 21]. Abnormalities of GAP activity of diagnostic value have been found in certain haematological diseases. In contrast to myeloid leukaemia where GAP activity is greatly reduced, myeloid reactions and polycythaemia are associated with an enhanced GAP activity. These figures are reliable enough to form a basis for routine investigations suited for differential diagnostic purposes [4, 8, 15, 16].

The values for GAP noted in Hodgkin's disease are greatly varying [3, 17], a fact connected by BENNETT [3] with the clinical course of the disease where remissions alternate with exacerbations. Increased values are thus interpreted by this author as a sign of impairment or even as a presage of recurrence.

In patients with Hodgkin's disease treated at our Department and its haematological clinic the possible relationship between GAP activity and the clinical stage of the disease has been studied with the aim of establishing

whether an increase in GAP activity may be regarded as predictive of a relapse. This would be of a practical significance, by indicating when to start therapy for the prevention of recurrences.

Material and method

All the patients involved in the study suffered from histologically verified Hodgkin's disease. The stage of the disease was classified according to Peters as follows:

Stage I = process confined to a sole lymph node group;
Stage II = involvement of more than one lymph node group but all of supradiaphragmatic site;
Stage III = subdiaphragmatic lymph node groups also involved;
Stage IV = generalization, infiltrative involvement of various organs.

Symptoms of systemic involvement at any time place the patient in the subsequent stage. Reliable classing to stage III may require the additional evidence of lymphography. This was actually performed in a fair number of patients belonging to stages I and II. Full remission was understood to mean a condition or a period marked by the absence of any sign or symptom; recurrence or exacerbation, the appearance of any of the following signs: enlargement of lymph nodes, spleen, liver, increase in BSR, fever, itching, pain, loss of weight, progressive anaemia. In stages III and IV there generally was some sign of activity it having been no longer possible to attain full remission in these stages.

The patients with evidence of activity in stages I and II were immediately started on massive-dose irradiation, supplemented in stages III and IV by cytostatic therapy. In stages III and IV where no full remission had been achieved, corticoid treatment was prescribed in the intervals.

Between January, 1968, and December, 1969, 20 patients with Hodgkin's disease were studied for GAP.

For the demonstration of GAP, Kaplow's azo-dye technique was used [2, 10, 13, 24]. In each case a total of 400 cells was counted, i.e. 2×200 cells or two separate slides. The individual cells were graded from 0 to 5 according to the intensity of GAP activity, as recommended by HEILMEYER:

0 = no demonstrable change;
1 = the cytoplasm shows barely discernible brownish patches;
2 = inhomogeneous dye deposits in the cytoplasm with the exception of the marginal parts;
3 = dye deposits over the entire cytoplasm, with the cellular structure still discernible in all its details;
4 = dye deposits forming black clumps through which the nucleus is faintly discernible;
5 = the entire cell is occupied by a black homogeneous mass.

The index given here refers to 100 cells. Normal values averaged 60 ± 21. Enzyme activity in smears of normal subjects hardly ever attained grade 4 or 5. Due attention was paid to intercurrent diseases, in which case GAP determinations were refrained from.

Results

The data grouped according to clinical stage and recurrence or remission are shown in Table I.

From Table I it emerges that in the absence of any clinical activity the GAP value was normal in every case but one. Moderate anaemia was present but BRS and leukocyte count were entirely normal. Patient K.J. relapsed one year later.

As Table II shows, in the cases marked by clinical activity the GAP value was usually increased. The highest levels were found in stage IV. It was

likewise in this group where the highest BSR values were demonstrable. In the smears of the subjects in stages III and IV, cells of grade 4 and 5 reflecting an excessive GAP-activity could be noted. The figures seen in Tables I and II

Table I

Data of patients in full remission

Name	Stage	GAP	BSR, mm	WBC	RBC, million
P.J.	I	77	16	5,600	3.5
V.A.	I	60	5	8,200	4.2
K.J.	I	120	6	7,200	3.4
P.J.	I	60	8	6,000	3.5
Sz.L.	I	78	16	6,000	4.1
B.B.	I	40	20	4,000	3.8
N.J.	I	66	12	4,800	3.1
K.K.	II	30	20	8,000	3.6

Table II

Data of patients during exacerbation

The data pertaining to stages I and II have been obtained prior to treatment. Those referring to stages III and IV represent two extreme values of repeated determinations

Name	Stage	GAP	BSR, mm	WBC	RBC, million
K.J.	I	190	120	8,000	3.4
Sz.L.	I	80	25	5,200	3.1
N.J.	I	80	25	6,000	4.5
P.I.	I	150	45	7,200	4.1
B.B.	I	120	48	8,400	4.2
M.M.	II	48	25	10,000	3.0
Sz.J.	II	30	58	6,600	3.6
K.K.	II	90	60	11,000	3.6
B.B.	II	134	32	4,000	3.4
B.L.	II	191	10	6,400	4.1
V.L.	III	120	100	6,300	3.9
K.J.	III	60—150	55—110	c. 7,000	c. 3.4
H.Zs.*	IV	160	100	10,000	2.8
B.I.*	IV	80—192	80—125	16,000	c. 3.5
U.L.*	IV	66—248	20— 99	c. 6,000	c. 3.5
T.L.	IV	70—276	20— 89	c. 8,000	c. 3.6

* Died

show a close relationship between the changes in GAP and in BSR but none between those of GAP and the RBC or WBC.

Table III also shows a certain parallelism between the changes in GAP and in BSR. Peak values usually occurred before or during treatment and normalized as a result of therapy.

Table III

GAP values in four patients at intervals of one month

Stage							
I		I		I		II	
GAP	BSR	GAP	BSR	GAP	BSR	GAP	BSR
120	*45*	36	50	*80*	*25*	90	60
61	20	*150*	*35*	60	12	*120*	*40*
40	20	80	8	40	20	60	20
80	14	80	12	119	22		
65	14	60	16				
40	12	90	14				
60	14						
Name							
B.B.		P.I.		N.J.		K.K.	

Periods of treatment are shown by italics

Discussion

The results of GAP studies in Hodgkin patients appear widely dissimilar if presented without order or system. If, however, they are grouped in accordance with the clinical stage or the degree of activity they reveal a definite relationship. Full clinical remission was usually associated with normal values whereas recurrences went hand in hand with an increase in the GAP value related to the severity of the clinical condition. In stages III and IV, normal GAP values were hardly ever found even during relative remissions. There was a definite parallelism between the changes in GAP and in BSR: both these values increased parallel with clinical deterioration. Serial studies allowed to follow the successive normalization of the GAP value under the influence of successful therapy. Since many of the patients had to travel from a great distance, during remissions they failed to present for monthly follow-up, therefore we lack sufficient evidence as regards the successive GAP-changes

accompanying or preceding recurrences. Nor were we able to ascertain how long before an actual clinical recurrence of the GAP activity had begun to rise. The present study has thus failed to provide an answer to the question whether elevated GAP values are really predictive of clinical recurrences in Hodgkin's disease. However, even the present scarce data left no doubt about the existence of close relationships between enhanced GAP activity and clinical deterioration. Still, the prognostic utilization of these relationships will remain largely a theoretical issue. Many centers lack facilities for repeated GAP estimations whereas the BSR which has much the same informative value, can be performed everywhere.

The proper evaluation of the GAP value in Hodgkin's disease depends on various factors. Not only is the procedure itself fraught with considerable sources of error in which subjectivity also plays a part but the disease under study and its therapy also involve various factors which may affect the activity of GAP, to quote only the type of treatment, the patient's hormonal and immunological state, possible intercurrent diseases, etc. But even if we should be able to rule out these factors and thus to connect the increased GAP value with a clinical deterioration, we are still in ignorance of the cause of the enhanced activity.

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SURGICAL OBSERVATIONS IN POLYCYTHAEMIA VERA PATIENTS

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(Received July 9, 1970)

On the evidence of surgical observations in 14 patients with polycythaemia vera full remission at the time of the intervention is regarded as the only possible safeguard against the postoperative hazards of haemorrhages and thromboembolism inherent in the disease.

Haemoblastoses are often associated with disturbances of blood coagulation which greatly add to the hazards of surgery in these diseases and raise special problems as regards the decision for the intervention including its timing and the necessity for preoperative and postoperative measures.

The incidence of thromboembolic and haemorrhagic complications in untreated or poorly controlled cases of polycythaemia vera is remarkably high [1, 5—8, 10, 13, 16]. CHIEVITZ and THIEDE [3], in a survey of 250 cases of polycythaemia vera found thrombosis, thromboembolism or haemorrhagic complications to be the direct cause of death in 50%. RIGBY and LEAVELL [18] noted the occurrence of thrombosis in 20 and of bleedings in 15 out of 50 cases. TCHERBAK [4] in a survey of 219 patients with polycythaemia vera followed up for more than ten years, noted the occurrence of thrombosis, bleedings or both in 139 or 63%. WATKINS and FAIRLEY [21] on the basis of published evidence and their own observations, estimated the incidence of vascular complications in polycythaemia vera at 38 to 67%. In their view it is owing to these complications interfering with adequate control that the survival in polycythaemia vera is as short as 18 months.

We have been concerned with the pathogenetic, clinical and therapeutic aspects of polycythaemia vera since 1959 and during this period 96 patients have been treated and followed up. Forty-six of them had had vascular complications, i.e. thrombosis, bleedings or both before admission, thus, before the start of active therapy.

During this period surgical interventions of some kind had to be performed in 14 cases. In view of the special problems involved by the high incidence of thromboembolic and haemorrhagic manifestations in polycythaemia vera adding to the surgical hazards of this disease it seemed of interest to give a summary of our observations.

Material and method

The 14 surgical cases included 8 males and 6 females aged between 26 and 75 years, with a mean of 55.5 years. The duration of the disease ranged between 3 and 8 years.

At the time of surgery, 11 patients were in full remission as a result of successful therapy and 3 were in a stage of exacerbation. In the patients in remission the erythrocyte counts averaged 4,180,000 per ml, the haematocrit 41%, the leukocyte counts 7,000 and the platelet counts 207,000 per ml. In the stage of exacerbation the values were: 6,800,000, 61%, 11,500 and 586,000, respectively.

It was always attempted to achieve full remission before surgery. In two of the three patients operated upon during a relapse the disease had remained unrecognized until after operation despite the high erythrocyte count and haematocrit values found preoperatively. Surgery had been performed elsewhere and the patients were referred to us afterwards for closer investigation. In the third case it was an acute abdomen which called for an emergency operation regardless of the activity of the process.

The 14 surgical interventions were: gastrectomy (1 case), repair of hernia (3 cases), nephrectomy (1 case), radical operation for mammary tumour (1 case), appendectomy (1 case), amputation of the toes of the right foot (1 case), splenectomy owing to excessive splenomegaly of rapid progression despite normal blood counts (1 case), exploratory laparotomy with liver biopsy (1 case), exploratory laparotomy with intestinal resection (1 case), cholecystectomy (1 case), eye surgery (2 cases) (Table I).

Results

Postoperative complications occurred in 6 patients of the series. All three patients operated on during relapse developed vascular complications, one (K.I. aged 26) an extensive haematoma of the abdominal wall with thrombosis of the leg; one (B.J. aged 56) thrombosis of the leg followed by pulmonary embolism; and one died of mesenterial thrombosis for which an intestinal resection had been performed. Of the 11 patients having had surgery during remission, three developed postoperative complications. Minor haematoma occurred in two (O.M. aged 56, and V.Gy, aged 44) and bleeding from the wound edges, readily controlled by mattress sutures, in one (S.J., 46-year-old female).

The patients having been in remission at the time of surgery had had ^{32}P or cytostatic therapy 4 to 6 months earlier, 3 had ^{32}P, 5 dibromomannitol and 3 tetramethylmannitol.

Two patients had extensive varicosities. Elastic bandages were applied in both for the postoperative period. Early mobilisation was performed in all of the cases.

Discussion

It is well known that the incidence of thromboembolism in the postsurgical period exceeds that found in the general population. The statistical figures show a fairly wide variety range between 0.6 and 3.5% [12, 20]. Haemorrhages, apart from these consecutive upon surgery, are confined to haemorrhagic diseases and/or disturbances of coagulation [2, 11].

The occurrence in polycythaemia vera of thromboembolism together with haemorrhagic manifestations still awaits elucidation. While increased

Table I

	Name	Age	Stage	R.B.C. million	Hcrit per cent	Platelets	Type of surgery	Complication	Wound healing
1.	O.M.	56	R	4.2	40	143,000	Gastrectomy	Abdominal wall haematoma	Moderately protracted
2.	Zs.I.	54	R	4.0	40	243,000	Herniotomy	Abdominal wall haematoma	First intention
3.	B.J.	73	R	4.0	38	200,000	Amputation of toes	Abdominal wall haematoma	First intention
4.	M.F.	53	R	4.2	41	277,000	Appendectomy	Abdominal wall haematoma	First intention
5.	K.P.	58	R	4.1	43	197,000	Mayo's operation (hernioplastica)	Abdominal wall haematoma	First intention
6.	Sz.K.	61	R	4.9	45	250,000	Repair of abdominal wall	Abdominal wall haematoma	First intention
7.	V.Gy.	44	R	4.9	45	380,000	Mammectomy for tumour	Haematoma	Moderately protracted
8.	K.I.	26	E	7.0	66	720,000	Exploratory laparotomy	Abdominal wall haematoma thrombosis	Protracted
9.	A.I.	67	R	4.6	42	100,000	Extraction of cataract	—	First intention
10.	G.F.	74	R	3.8	36	135,000	Extraction of cataract	—	First intention
11.	B.J.	56	E	6.7	63	650,000	Nephrectomy, left side	Haemorrhage thrombosis extr. inf. l. d. pulmonary embolism	Protracted
12.	H.J.	49	R	3.9	41	207,000	Cholecystectomy	—	First intention
13.	S.J.	46	R	3.8	40	145,000	Splenectomy	Haemorrhage	Moderately protracted
14.	S.S.	60	E	6.6	54	390,000	Extirpation of lipoma of thoracic wall	Resection of small intestine for mesenteric thrombosis two weeks later	

blood viscosity, slowing of circulation and thrombocythaemia seem to account for the hazards of thrombosis, the haemorrhagic manifestations are ascribed to a distension of the capillary wall and to abnormalities of the individual plasma factors [10]. The prevalence of the disease in or beyond the age groups of 40—50 years is suggestive of a possible involvement of atherosclerotic changes of the vessel wall.

According to the studies of NAGY et al. [14] the most consistent abnormalities of the coagulogram were in the prothrombin index, prothrombin consumption, thrombin inactivation and the platelet count. In a number of cases, bleeding time, recalcification time, thrombin and toluidine blue times also revealed abnormalities. The alterations of the two last-named parameters raise the possibility of an accumulation of circulating anticoagulants. KOLUTOVA [8], LASCH and LINKE [9] regard hyperheparinaemia which they demonstrated in patients with polycythaemia vera as one of the factors, if not as the only cause, of the haemorrhagic manifestations, the more so as nearly full normalization of these changes was noted parallel with remissions. The thromboelastographic studies performed by NAGY et al. [15], and NAGY and BURGER [17] revealed abnormalities involving reaction and coagulation times, maximum elasticity and fibrinolysis; these parameters showed little improvement during remissions, a fact which may account for the possible, though rare, occurrence of complications during remissions.

Since polycythaemia vera is incompatible with the usual measures serving for the prevention of haemorrhagic or thromboembolic manifestations, the only way of avoiding surgical complications of this kind in polycythaemic patients is to refrain from operation until complete remission has been achieved. This is clearly illustrated by the occurrence of vascular complications in all three cases where surgery had been performed during exacerbation and their absence in all but three of the 11 patients operated upon in full remission. Also, while the complications occurring during exacerbations were lethal or grave, those appearing during remissions were slight and reversible.

We were unable to trace more than a few sporadic observations on the subject under discussion in the literature. TINNEY et al. [19] in a series of 163 patients with polycythaemia vera noted haemorrhagic complications in 53 cases, in 17 cases after surgery, particularly after dental extractions (1943). Of the 50 patients reviewed by RIGBY and LEAVELL [18], 15 had been subjected to major or minor surgery; significant postoperative bleeding or thrombosis occurred in 5 of these.

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LIPOLYTIC ACTIVITY OF EPINEPHRINE ON HUMAN SUBCUTANEOUS ADIPOSE TISSUE OF OBESE AND LEAN PERSONS IN VITRO

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Spontaneous lipolysis and the effect of epinephrine on subcutaneous adipose tissue of 45 normal weight and 29 obese persons was examined *in vitro*.

(1) Spontaneous lipolysis of adipose tissue of normal weight and of obese subjects was identical.

(2) Epinephrine similarly and significantly increased FFA-release in both groups.

(3) In spontaneous and epinephrine-induced lipolysis there was no sex difference among the persons of normal weight, but in the obese group the adipose tissue of males reacted poorly to epinephrine.

(4) In both groups the subjects under forty years of age showed a decreased spontaneous release of FFA, but a more intensive response to epinephrine in comparison with the older subjects; the difference was significant only in the obese group.

Introduction

Adipose tissue has widely been examined for its lipid metabolism in the last decade. GORDON and CHERKES (1958) were the first to investigate *in vitro* the effect on rat adipose tissue of the best known lipolytic hormones, epinephrine and ACTH. Subsequently it was found that the adipose tissue of obese rats was less sensitive to lipolytic effects than that of animals of normal weight (LEBOEF et al. 1961, MAYER 1965).

Reports on similar investigations in human adipose tissue are conflicting. HIRSCH and GOLDRICH (1965) found no difference in FFA-release between normal weight and overweight patients. LASZLO (1965) showed that the subcutaneous adipose tissue of extremely obese patients mobilized less FFA than did that of lean persons. Norepinephrine was found to increase FFA-release by the adipose tissue of normal and obese subjects. ÖSTMAN (1965) reported that mesenteric adipose tissue was more sensitive to lipolytic effects than subcutaneous adipose tissue; there was no difference in spontaneous FFA-release by subcutaneous adipose tissue between normal weight juveniles and obese elderly persons. Nor did MOSINGER et al. (1965) find differences in spontaneous FFA-release by subcutaneous adipose tissue between normal and obese persons. In response to epinephrine, FFA-release was moderately increased in both groups. Mesenteric adipose tissue of obese patients was more sensitive to,

and its response was more intensive after, epinephrine treatment. GALTON and BRAY (1966) found no difference between lean and obese subjects in spontaneous and epinephrine-induced lipolysis in isolated subcutaneous adipose tissue cells.

All the above investigations were carried out on a small number of subjects (not exceeding ten) and by different methods. In the present study, spontaneous FFA-release of human subcutaneous adipose tissue and its response to small doses of epinephrine has been investigated in vitro in a number of persons of normal weight and in obese individuals.

Material and methods

Subcutaneous adipose tissue was obtained from 45 persons of normal weight, displaying no metabolic disorder, and from 29 obese patients, suffering neither of endocrine disease, nor of manifest diabetes mellitus. The tissue was removed from the abdominal subcutaneous fat layer surgically or by biopsy. Patients whose weight exceeded 20 kg the number of cm above 100 cm of their length, were regarded as obese. The operation was performed in general anaesthesia except for 5 cases where it was carried out in anaesthesia with 0.5% procaine. In cases of biopsy, the following method of local anaesthesia was used. A rhombic 5 cm by 5 cm infiltration was made on the left abdominal side with 1% xylocaine and the 3 cm incision line in the middle of the rhombus was infiltrated intradermally. The removed 2—3 g adipose tissue remained free from the anaesthetic solution. Wound healing, except for a small haematoma in two cases, was uneventful. Prior to the investigation all patients were given a diet containing at least 2000 Cal with 200 g carbohydrate. On the morning of the intervention they were fasting.

The adipose tissue was placed into Krebs—Ringer-bicarbonate buffer at room temperature and subsequently minced into 20 to 40 mg pieces. About 100 mg of adipose tissue was placed in Hagedorn's tubes into 3 ml Krebs—Ringer-bicarbonate buffer containing 2% lyophilized human albumin (Research Institute for Human Vaccines, Budapest). Incubation was carried out at pH 7.4 at 37 °C temperature for two hours in a metabolic shaker at 90/min oscillation frequency under air; 3 μg/ml of epinephrine was added to other flasks. Two, occasionally three specimens were examined in parallel. To assess lipolysis, the initial and postincubation FFA content of the medium was determined according to Dole (1956), and from the difference the released fatty acid was calculated in μEq/g adipose tissue /2 hours. Student's t test was used for statistical analysis.

Results

The rate of spontaneous FFA-release by subcutaneous adipose tissue was similar in the normal and in the overweight subjects (Tables I, II, III). The anaesthesia did not influence lipolysis. There was a significant increase in FFA-release following epinephrine administration in both normal and obese subjects. At the same time, the adipose tissue of normal persons was somewhat more sensitive to epinephrine than that of obese subjects (Table III).

No sex difference was found in the normal group, either in spontaneous or in epinephrine-induced lipolysis. There was no sex difference in spontaneous FFA-release in the obese group, but the adipose tissue of obese males did not respond significantly to epinephrine stimulation (Table III).

Table I

Spontaneous and epinephrine-induced FFA-release by subcutaneous adipose tissue of normal subjects

Number	Sex	Age	Weight—length (kg)	Diagnosis	FFA-release (μEq/g/2 hrs)	
					Spontaneous	Epinephrine (3 μg)
1	M	59	+ 2	Rectal tumour	3.8	+2.2
2	M	45	+ 6	Inguinal hernia	3.8	+5.2
3	M	63	+ 9	Inguinal hernia	3.4	+3.4
4	M	28	− 9	Duodenal ulcer	1.6	+2.2
5	M	52	− 4	Abdominal hernia	3.0	−1.4
6	M	56	+10	Ureteral calculus	2.6	+1.8
7	M	24	− 9	Duodenal ulcer	2.3	+0.5
8	M	62	+11	Vesical papilloma	3.6	+1.5
9	M	61	+ 4	Inguinal hernia	1.5	+0.5
10	M	65	+10	Prostatic adenoma	2.5	+2.5
11	M	64	+ 4	Prostatic adenoma	4.0	+0.8
12	M	66	+ 8	Prostatic adenoma	1.1	+1.5
13	M	74	−13	Gastric ulcer	1.0	+2.1
14	M	47	+ 7	Pancreatic tumour	4.5	+0.8
15	M	57	−13	Abdominal hernia	2.9	+0.5
16	M	46	0	Gastric ulcer	0.5	+1.3
17	M	57	+ 5	Abdominal hernia	2.6	+1.1
18	M	54	+ 5	Abdominal hernia	3.5	+1.1
19	M	56	−13	Duodenal ulcer	2.0	+0.2
20	M	71	− 2	Prostatic adenoma	1.2	+0.6
21	M	67	+ 2	Cholelithiasis	2.0	+0.3
22	M	66	− 5	Gastric ulcer	4.6	+0.5
23	F	20	− 4	Inguinal hernia	1.8	+0.8
24	F	22	+ 2	Abdominal hernia	0.2	+2.0
25	F	32	+ 3	Abdominal hernia	1.8	+4.6
26	F	64	− 7	Inguinal hernia	1.8	+2.8
27	F	71	− 4	Gastric ulcer	1.6	+0.4
28	F	52	+17	Abdominal hernia	2.0	+2.8
29	F	50	+ 5	Cholelithiasis	2.2	+0.4
30	F	65	− 7	Cholelithiasis	3.8	+1.4
31	F	29	− 1	Cholelithiasis	3.2	+1.2
32	F	37	− 3	Abdominal hernia	4.2	+1.2
33	F	42	+ 8	Abdominal hernia	4.9	+2.6
34	F	37	0	Appendicitis	2.9	+1.8
35	F	23	+16	Appendicitis	1.5	+3.3
36	F	57	+ 7	Ureteral calculus	5.8	+1.6
37	F	43	+ 5	Renal calculus	0.6	+1.0
38	F	70	− 5	Colon tumour	4.5	+0.6
39	F	47	+ 1	Abdominal hernia	3.5	+2.2
40	F	38	+13	Cholelithiasis	1.9	+0.5
41	F	35	− 8	Gastric ulcer	3.4	+2.3
42	F	61	− 4	Renal calculus	3.6	+0.3
43	F	69	− 6	Gastric tumour	3.3	+1.0
44	F	66	+10	Abdominal hernia	2.1	+0.7
45	F	18	+ 7	Appendicitis	1.7	+2.1

Sex and age of donors, weight kg − (length cm − 100), diagnosis, spontaneous FFA-release and response to 3 μg/ml epinephrine are given. The latter means the plus lipolysis related to spontaneous lipolysis. Tissue was removed surgically under general anaesthesia, except in patients Nos 2, 3, 9, 23, and 26, who were operated under local anaesthesia. Incubation was carried out for 2 hrs.

Table II

Spontaneous and epinephrine-induced FFA-release by subcutaneous adipose tissue of obese patients

Number	Sex	Age	Weight—length (kg)	Diagnosis	FFA-release (μEq/g/2 hrs)	
					Spontaneous	Epinephrine (3 μg)
1	M	46	+62	Obesity	3.6	−1.6
2	M	23	+58	Obesity	1.4	+0.4
3	M	56	+27	Cholelithiasis	3.2	+0.6
4	M	31	+39	Obesity	1.7	+0.7
5	M	27	+45	Obesity	2.1	+0.8
6	M	38	+35	Abdominal hernia	3.3	+0.4
7	M	39	+41	Obesity	2.3	+0.8
8	M	25	+23	Obesity	1.7	+1.6
9	M	46	+36	Obesity	1.8	−0.5
10	M	53	+57	Obesity	5.8	+0.7
11	M	59	+77	Obesity	3.8	−2.8
12	M	22	+58	Obesity	2.5	+4.1
13	F	46	+36	Obesity	3.4	+0.6
14	F	29	+38	Obesity	3.0	+1.3
15	F	41	+47	Obesity	2.9	+0.6
16	F	46	+39	Obesity	3.1	+0.5
17	F	30	+37	Obesity	2.9	+0.7
18	F	42	+36	Obesity	2.6	+0.2
19	F	34	+25	Obesity	2.3	+2.8
20	F	25	+22	Obesity	3.8	+1.2
21	F	47	+21	Obesity	3.5	+0.3
22	F	42	+52	Obesity	3.8	+2.0
23	F	56	+43	Obesity	2.8	+3.8
24	F	23	+48	Obesity	2 0	+1.7
25	F	15	+47	Obesity	3.2	+2.4
26	F	45	+23	Obesity	3.4	+0.4
27	F	37	+46	Obesity	0.8	+1.0
28	F	43	+29	Abdominal hernia	3.3	+0.5
29	F	34	+29	Obesity	1.6	+2.2

Sex and age of donors, weight kg − (length cm − 100), diagnosis, spontaneous FFA-release and response to 3 μg/ml epinephrine are given. The latter means the plus lipolysis related to spontaneous lipolysis. The tissue was removed by biopsy except in patients Nos 3, 6, and 28, who were operated under general anaesthesia. Incubation was carried out for 2 hrs.

In both the normal and the obese groups, the adipose tissue of patients under 40 years of age showed a decreased spontaneous FFA release but a more intensive response to epinephrine, than that of older subjects. The difference was significant only in the obese group (Table III).

Table III

Spontaneous and epinephrine-induced FFA-release by subcutaneous adipose tissue of normal and obese subjects. Sex and age differences

		n	FFA-release (μEq/g/2 hrs; mean $\pm$ S.E. of mean)		p
			Spontaneous	Epinephrine (3 μg)	
Normal weight		45	2.67$\pm$0.18	+1.48$\pm$0.18	$<$0.001
Obese		29	2.81$\pm$0.21	+0.94$\pm$0.26	$<$0.01
Normal weight	males	22	2.63$\pm$0.25	+1.32$\pm$0.28	$<$0.001
	females	23	2.71$\pm$0.29	+1.63$\pm$0.22	$<$0.001
Obese	males	12	2.77$\pm$0.45	+0.43$\pm$0.49	N.S.
	females	17	2.84$\pm$0.30	+1.30$\pm$0.24	$<$0.001
Normal weight	under 40 years	12	2.22$\pm$0.30	+1.89$\pm$0.34	$<$0.001
	above 40 years	33	2.87$\pm$0.23	+1.34$\pm$0.20	$<$0.001
Obese	under 40 years	15	2.30$\pm$0.35*	+1.47$\pm$0.28**	$<$0.001
	above 40 years	14	3.35$\pm$0.23*	+0.38$\pm$0.40**	N.S.

* and **$p<0.05$ in comparison with each other. N. S. = not significant.

Number of cases, spontaneous FFA-release and the response to 3 μg/ml epinephrine are given. The latter means the plus lipolysis related to spontaneous lipolysis. Incubations were carried out for 2 hrs and the results expressed as the mean $\pm$ S.E.M. P values of epinephrine response.

Discussion

Our findings concerning the rate of spontaneous lipolysis of human adipose tissue are in accordance with those reported in the literature. LASZLO (1965) found a higher spontaneous FFA-release in normal subjects than we did. This author, however, used as medium the patient's own plasma containing hormones and other factors influencing fat mobilization. We found (unpublished data) that the fatty acid mobilizing activity of the plasma of obese persons is reduced. Also our values for the stimulating effect of epinephrine differ from those reported by MOSINGER et al. (1965); these authors found a moderate response to epinephrine of the subcutaneous adipose tissue of both normal and obese patients, while a significantly higher FFA-release by the mesenteric adipose tissue of overweight patients. The different behaviour of subcutaneous and mesenteric adipose tissue is well-known. Our findings differ also from those reported by ÖSTMAN (1965) in that the adipose tissue of our older obese patients showed a higher spontaneous FFA release than that of normal juveniles.

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SERUM HAPTOGLOBIN LEVEL IN ATHEROSCLEROSIS

By

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The serum haptoglobin level had been studied in 387 atherosclerotic subjects with myocardial infarction, coronary sclerosis, acute cerebro-vascular insults, peripheral arterial disease. A significant increase in the level was demonstrable in all these conditions, particularly in acute complications such as myocardial infarction and cerebro-vascular insults. Association of diabetes with atherosclerosis did not seem to affect the haptoglobin level. No correlation was demonstrable between the serum haptoglobin and cholesterol levels. In 25 subjects with acute myocardial infarction the peak haptoglobin was attained by the end of the third week.

Haptoglobin, a glycoprotein forming a firm complex with haemoglobin, was first described by POLONOVSKI and JAYLE [23]. SMITHIES and WALKER [26, 27] separated it into three types constituting a system of hereditary character. The haptoglobin level is fairly constant in the same individual [18, 19] but varies widely in different subjects. According to NYMAN [19], the mean level amounts to 110 mg per 100 ml (range, 30 to 190 mg per 100 ml). This value agrees with those observed by other authors [5, 9, 21, 25]. NYMAN [19] found slightly higher values in males than in females, while HEVÉR [5] and SHINTON et al. [25] failed to demonstrate any significant sex difference. Nor has ageing any demonstrable influence on the haptoglobin level [15, 19, 25]. The values are slightly affected by the type of haptoglobin; this is, however, usually disregarded, owing to the wide normal range.

The clinical significance of haptoglobin has been attracting increasing interest in recent years. Its level has been found to increase in various processes associated with the breakdown or reorganization of tissues, in particular in acute and chronic inflammatory conditions, numerous infectious diseases, collagen diseases, tumours, etc. [4, 5, 9, 19]. Increased values were found in various non-haemolytic diseases of the haemopoietic system, e.g. in panmyelopathy [12, 17], Hodgkins's disease, reticuloses [6, 11, 13], though not in haemoblastoses where the figures are inconsistent [6, 12, 21]. On the other hand, in haemolytic processes the haptoglobin level is considerably depressed [9, 12, 19, 21, 22, 25], a finding characteristic of every haemolytic reaction, and thus of diagnostic importance in the case of complications after blood transfusion [2, 15].

JAYLE et al. [7, 9, 10] found a high haptoglobin level in coronary disease and its further rise after myocardial infarction. There is also other published evidence on increased haptoglobin values in coronary thrombosis [1, 14, 16, 22], though MÜLLER and MÜLLER-VON VOIGT [18] failed to demonstrate any significant elevation in such cases.

In view of the qualitative and quantitative alterations of serum mucopolysaccharides in atherosclerosis, the above observations seemed to justify a study of the behaviour of haptoglobin, a substance composed of protein and polysaccharide, in some conditions associated with atherosclerosis.

Material and methods

a) Patients

The haptoglobin level was determined in 612 subjects with atherosclerosis of various types and severity. In the majority a single estimation was performed, in some patients two or three estimations, and in 25 patients with myocardial infarction the haptoglobin level was recorded serially. In the interest of correct interpretation, any potential additional factor was ruled out which might have affected the haptoglobin level. The cases where this was not possible with any reliable certainty, were excluded from analysis. In this manner, the data of 387 patients were available for processing. There were 180 males and 207 females, aged between 37 and 88 years (mean 66 years). The patients were divided into five clinical groups on the basis of the following criteria.

1) *Myocardial infarction.* Typical course, laboratory findings, ECG; less than 4 weeks since the infarction.

2) *Coronary sclerosis.* Typical anginal symptoms, ECG, and, in a number of cases, a history of myocardial infarction.

3) *Acute cerebro-vascular insult.* Patients in the first week after the onset of hemiplegia, aphasia or of other focal lesions, whether proving transitory or permanent in the end. Carotography was done in a number of cases.

4) *Cerebrosclerosis.* One or several cerebrovascular insults in the past, mentioned in the history or diagnosed on the basis of hemiplegia or of other focal lesion.

5) *Peripheral arterial disease.* Typical signs and symptoms, including oscillographic pattern, with no evidence of necrosis.

Group 1 comprised 67 subjects (41 males, 26 females); group 2, 205 subjects (81 males, 124 females); group 3, 48 subjects (21 males, 27 females); group 4, 52 subjects (24 males, 28 females); group 5, 15 subjects (13 males, 2 females).

82 patients (30 males, 52 females) had also diabetes. Their data were evaluated separately in the respective groups.

As a control group, 52 healthy blood donors (36 males, 16 females) were used. The controls for the patients with atherosclerosis and diabetes were 10 young diabetics (5 males, 5 females), with no vascular complication.

b) Methods of investigation

The serum level was estimated by the colorimetric procedure of OWEN et al. [20] based on the measurement of haptoglobin methaemoglobin peroxidase activity. All tests were performed against controls for which the same mixture of normal sera with less than 5% scattering was used. The haptoglobin value was computed from a calibration graph and expressed in mg per 100 ml of bound methaemoglobin. The normal range was between 40 and 190 mg per 100 ml.

The serum cholesterol level was estimated by the method of RAPPAPORT and EICHHORN [24].

In the first group (myocardial infarction) SGOT, LDH, ESR and WBC were also estimated.

Results

Fig. 1 shows the haptoglobin values in the entire material. In the great majority of cases the level was between the mean and the upper normal limit. Table I shows the figures obtained in the controls, Table II those ob-

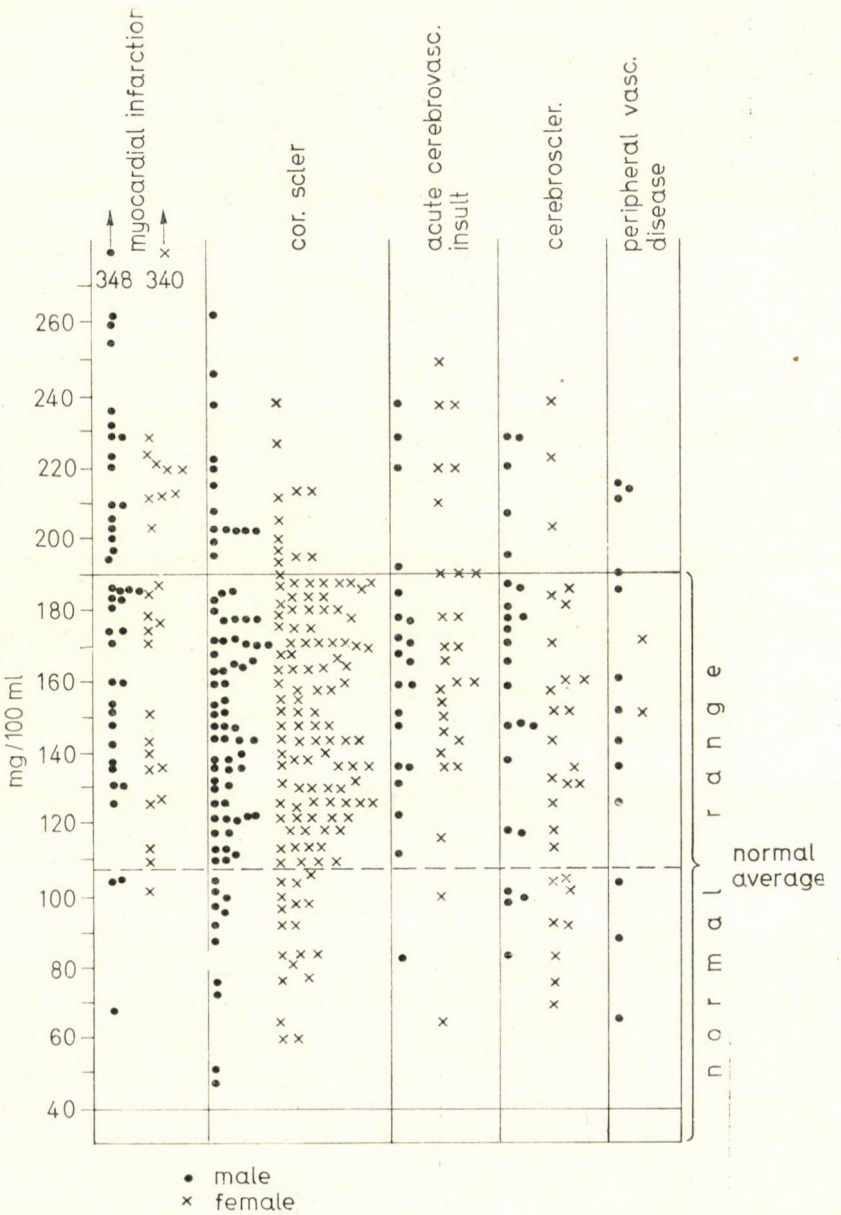

Fig. 1. Serum haptoglobin values in the various forms of atherosclerosis

Table I

Serum haptoglobin level, mg per 100 ml, in control subjects

	Normal blood donors			Diabetic controls			Total of controls		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
n	36	16	52	5	5	10	41	21	62
$\bar{x}$	109	105	108	141	139	140	113	113	113
s	34	35	34	43	52	45	35	38	38
$s_{\bar{x}}$	5.7	8.7	4.7	19.2	23.2	14.2	5.4	8.3	4.8

tained in the patients. Statistical significance was evaluated by the two-sample *t* test.

The data, whether referred to the total number of males or to that of females or to both sexes, consistently revealed a significant elevation of the serum haptoglobin level in all five groups (Tables I and II). To clarify the question whether or not the haptoglobin level is affected by diabetes associated with atherosclerosis, the values for the non-diabetic subjects were compared with those for the diabetics, with respect to sex and clinical type. No significant difference was found between the diabetic and non-diabetic groups. In conformity with this, the four-sample *t* test also failed to reveal any significant difference in the case of diabetes associated with atherosclerosis.

The cholesterol level was significantly increased in all groups, but correlation analysis failed to reveal any relationship between the haptoglobin and cholesterol levels.

In the next series, the serum haptoglobin level was serially recorded in 25 cases of myocardial infarction. The correlation was tested on the basis of a regression line derived from the individual values with the smallest scatter. This resulted in the parabola seen in Fig. 2 which shows that the average peak of 180 mg per 100 ml was reached towards the end of the third week.

Table II

Serum haptoglobin level, mg per 100 ml, in atherosclerosis,

	Myocardial infarction			Coronary sclerosis		
	Male	Female	Total	Male	Female	Total
n	41	26	67	81	124	205
$\bar{x}$	185	179	183	151	151	151
s	51	52	51	43	37	39
$s_{\bar{x}}$	7.9	10.1	6.2	4.7	3.3	2.7
t	7.50	4.84	8.75	4.97	4.35	6.26
p%	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

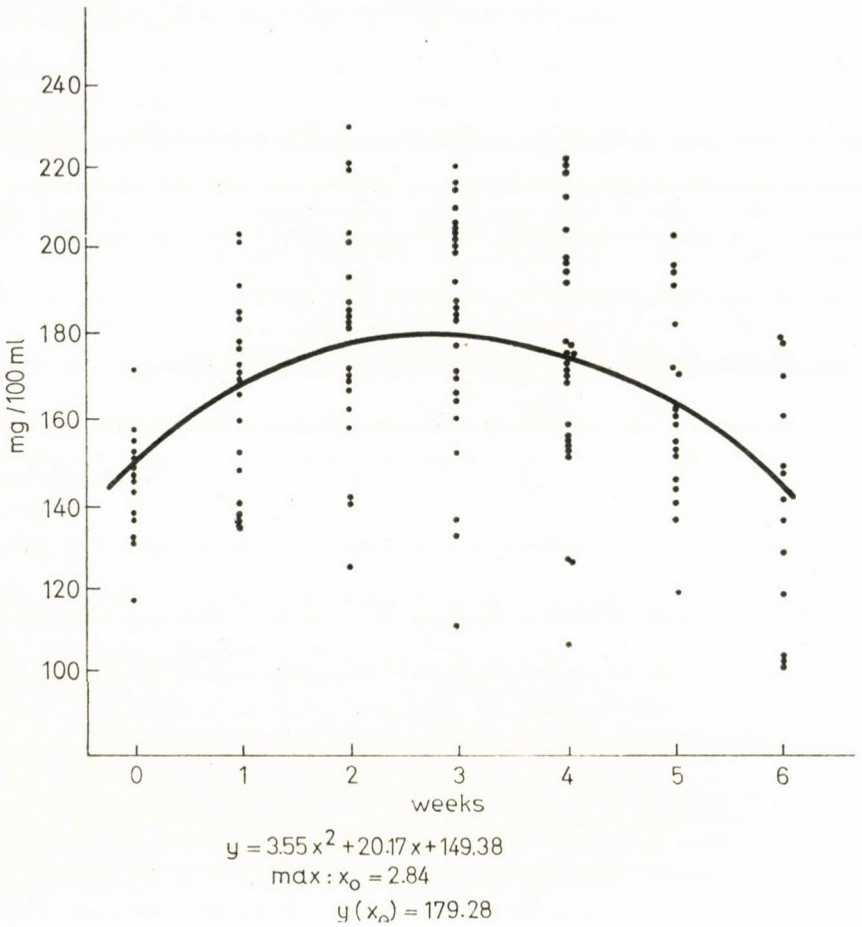

Fig. 2. Regression parabola of the serum haptoglobin level in myocardial infarction

including diabetic and non-diabetic subjects

Acute cerebro-vascular insult			Cerebrosclerosis			Peripheral arterial disease		
Male	Female	Total	Male	Female	Total	Male	Female	Total
21	27	48	24	28	52	13	2	15
156	170	167	161	139	150	149	—	154
33	43	40	42	40	44	46	—	36
7.9	8.2	5.8	8.5	8.1	6.1	15.2	—	11.8
4.39	4.71	7.23	4.97	2.25	4 82	2.63	—	3.58
<0.1	<0.1	<0.1	<0.1	<5	<0.1	<5	—	<0.1

Finally, it was studied whether the serum haptoglobin level in myocardial infarction was correlated with the SGOT, LDH, ESR or WBC values. The correlation coefficient revealed a slightly positive correlation between haptoglobin and SGOT during the first days after infarction, and also between haptoglobin and LDH one week after infarction. The subsequent data were insufficient for evaluation. Haptoglobin and ESR values showed a definite correlation at the end of the first and the second week. The correlations between the previous and later values were less definite. No correlation was demonstrable between haptoglobin and WBC.

Discussion

The present findings have shown a significant increase in the serum haptoglobin level in all the studied types of atherosclerosis. These findings agree well with the data according to which any process accompanied by tissue injury involves a rise in the haptoglobin level, and which would also account for our finding that the highest elevation occurred in the groups marked by grave tissue destruction; in acute complications, namely in myocardial infarction and cerebrovascular insult.

It has been sought to clarify also the question whether sex affects the behaviour of the haptoglobin level. The mean values displayed no sex-related difference; there were slight deviations in the values in the individual groups, but these were likely to occur in either direction.

In our patients, diabetes associated with atherosclerosis had no influence on the haptoglobin level. Though each of the two conditions involves a rise in the haptoglobin value, this influence does not seem to be additive in the case of their association.

As to myocardial infarction, this was the condition in the present study where the highest levels (mean 180 mg per 100 ml) were found; they occurred by the end of the third week. In cases where there is strong suggestive, but no conclusive, evidence of myocardial infarction, the haptoglobin level may provide diagnostic aid. This may be true to a certain extent for the other clinical types of atherosclerosis, too, but in these conditions there is a wide range of other signs and symptoms which are of higher diagnostic value.

In the patients with myocardial infarction the peak haptoglobin value was attained slightly later in time than it had been expected on the basis of the data in literature [1, 14, 16, 22], though these too are inconsistent. At any rate, here the behaviour of haptoglobin seems to follow a definite pattern. According to the present cases, it seems to correspond to a shallow parabola with a return to the initial value in the 5th or 6th week, rather than to a graph with a steep upward initial slope.

Slight correlations have been noted between the haptoglobin and SGOT levels in the first days after acute myocardial infarction. In the light of the above findings, the reason for this seems obvious. At the end of the first 48 hours when SGOT has reached its peak and is rapidly falling to its normal value, the concentration of haptoglobin only begins to rise. The correlation demonstrable between haptoglobin and LDH a week after myocardial infarction may be explained by a joint rise in the two values in this particular period. The correlations between ESR and haptoglobin at the end of the first as well as of the second week, are self-explanatory, since the ESR value increases together with the haptoglobin level in the first two weeks, but the ESR generally declines afterward.

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X-RAY FEATURES OF RENAL DISPLACEMENT AND COMPRESSION BY ENGLARGED SPLEEN AND LIVER IN POLYCYTHAEMIA VERA

By

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The roentgenologic appearance of the kidney was studied in 30 patients with polycythaemia vera. Displacement of the left kidney was demonstrable in 28, of the right kidney in 17 cases. The extent of renal displacement was related to the clinical activity of the disease and to the degree of spleno- and hepatomegaly. The roentgenologic signs of renal displacement during the periods of recurrence showed a definite regression during remissions.

Classification of polycythaemia on aetiopathogenetic grounds is in conformity with the clinical appearance of the condition [12]. The choice of treatment as well as prognostic considerations make it important to differentiate carefully between the individual types of the condition.

Polycythaemia vera belongs to the myeloproliferative diseases [3] marked by an overall proliferation of the haematopoietic system, first of all of the erythropoietic and, to a lesser degree, of the myelo- and thrombopoietic system [8, 9, 10, 11].

While untreated or poorly controlled cases of polycythaemia vera carry a high incidence of vascular complications involving irreversible consequences or acute life-threatening complications [11], adequate therapy considerably improves the prognosis by ensuring long periods of remission, usually with fully preserved working capacity [13, 20].

Renal polycythaemia constitutes a particular type of secondary polycythaemia, appearing usually in association with renal tumours of various kinds, with polycystic kidney or with unilocular or multilocular cysts of the organ [2, 14, 18]. These complications are unresponsive to radiophosphorus or to cytostatics administered with benefit in polycythaemia vera [18]. On the other hand, unless the primary process is not bilateral, removal of the affected kidney ensures a permanent curve of polycythaemia [14].

Polycythaemia vera is usually accompanied by some degree of splenomegaly or hepatomegaly [8—11] which, as it is known from the literature [5, 6, 7, 21], may produce certain roentgenologic abnormalities of the kidney suggestive at first of some primary renal disease.

The present study has been concerned with the incidence of roentgenologic abnormalities of the kidney in polycythaemia vera, with the nature of

these abnormal features and their relationship with the haemotological status including the degree of hepatic and splenic enlargement. It also seemed of interest to study whether or not the changes in question were reversible.

Material and methods

In the studies, 30 patients with reliably diagnosed polycythaemia vera were involved, 16 males and 14 females between 27 and 68 years (mean, 51 years) of age. The diagnosis had been ascertained histologically in bone marrow biopsy material [11]. At the time of the study, 25 patients were in the stage of recurrence, 5 in remission.

The patients in recurrence had the following average values: erythrocytes 6,300,000 (5,730,000 to 6,660,000), leukocytes 12,700 (9,000 to 14,800), platelets 715,000 (510,000 to 1,100,000). In those being in remission, the erythrocyte, leukocyte and platelet counts were within the normal range. Of the 30 patients, 29 had some degree of splenomegaly, 27 had hepatomegaly.

Excretion urography was performed in every patient after appropriate preparation and it was repeated a few months later in 9 cases. Five patients in whom urography was performed repeatedly, had been in a state of recurrence at the first and in remission at the second examination, while in three patients the first examination was done during remission and the second during recurrence. In one patient, while being in remission and displaying normal blood counts after repeated courses of ^{32}P, the spleen gradually grew in size until its lower pole had attained the iliac crest In this patient, excretion urography was performed three times; the first when the spleen was still moderately enlarged, the second when enlargment of the spleen was at its highest; and the third after splenectomy.

Urography was combined with tomography in 14 cases and it had to be completed by perfusion urography in 4 and with aortography in 2 cases. The X-rays had to be faultless to be evaluated, otherwise the examination was repeated after the necessary preparation two or three days later. Urographs repeated for technical reasons were evaluated as single examinations.

Results

The size of the spleen and of the liver can readily be estimated by manual palpation and from the antero-posterior X-rays. According to RÖSCH [16], the spleen measures 3 to 7 cm in its transversal and 8 to 14 cm in its longitudinal diameter. In the present material, splenomegaly and hepatomegaly were graded on the grounds of the physical and X-ray signs as follows. Distinct (+) in 7 cases; moderate (++) in 13 cases; and marked (+++) in 9 cases. In grade + the lower border of the liver or of the spleen was palpable 1.5 fingerbreadths, in grade ++ 2 to 3 fingerbreadths, in grade +++ more than 3 fingerbreadths below the costal arch.

The roentgenologic kidney abnormalities are presented in Table I. Displacement and rotation of the right and of the left kidney as also other signs indirectly related or unrelated to splenomegaly or hepatomegaly are found under separate headings.

As seen in Table I, displacement of the left kidney was demonstrable in 28 and its rotation in 9 cases. The latter abnormality was associated with a displacement of the kidney in 8 of the 9 cases, in all of which splenomegaly was of a major degree. Displacement of the right kidney was present in 17 and

its rotation in 5 cases. Rotation was always associated with displacement. Marked hepatomegaly was present in all 7 cases of combined displacement of the right kidney.

Table I

Side	Cranial	Displacement			Total	Rotation	Other abnormalities
		Caudal	Medial	Caudal + medial			
Left	1	12	6	9	28	9	7
Right	1	7	2	7	17	5	10

The other renal abnormalities are seen in Table II, which shows that bilateral pyelectasis and pyelonephritis were found in two instances each. Renal calculosis was demonstrated in three cases. These complications existed side by side with marked splenomegaly and hepatomegaly.

Table II

Side	Pyelectasy	Pyelonephritis	Nephrolithiasis	Renal cyst	Lipomatosis	Duplicity of renal pelvis	Bulging renal contours	Total
Left	2	2	1	—	1	—	1	7
Right	2	2	2	2	1	1	—	10

In the majority of the present cases the X-ray abnormalities were characteristic of a caudal or of a combined, i.e. caudo-medial, displacement of the kidney. The most typical findings are shown in the following case reports.

Case 1. (Figs. 1a and b), H. F., a 56-year-old male. Both kidneys show normal excretion and concentration. The left renal shadow is seen approximately 4 cm below its normal level, with the lower pole in the height of the iliac crest. The contours of its lateral upper segment are straightened out by the considerably enlarged spleen being in its close proximity.

Case 2. (Figs 2a and b), D. M., a 62-year-old female. Both kidneys excrete at a normal rhythm and concentrate adequately. The lower pole of the left kidney reaches 4 fingerbreadths below the line of the iliac crest, being displaced 8 cm downward and 4 cm medially. It joins the homogeneous shadow of the enlarged spleen. The renal pelvis and the calices are distended. There is an intrapelvic staghorn calculus. The lower pole of the right kidney reaches 3 fingerbreadths below the iliac crest, being displaced downward by the enlarged liver. There is a moderate distension of the pelvic system.

Excretion urography was repeated a few months later in 9 cases; in 5 patients the first examination was done during a recurrence and the second during remission. In two patients a considerable, in three a complete, regression of renal displacement was demonstrable at the second examination. On the other hand, in those three patients who had been in remission at the time of the first and in exacerbation at the time of the second examination, a definite progression of renal displacement was demonstrable on the second occasion, associated with rotation in 2 cases.

One of the patients was in remission when first examined as well as at the times of the two subsequent follow-up studies. However, despite normal blood counts the spleen gradually

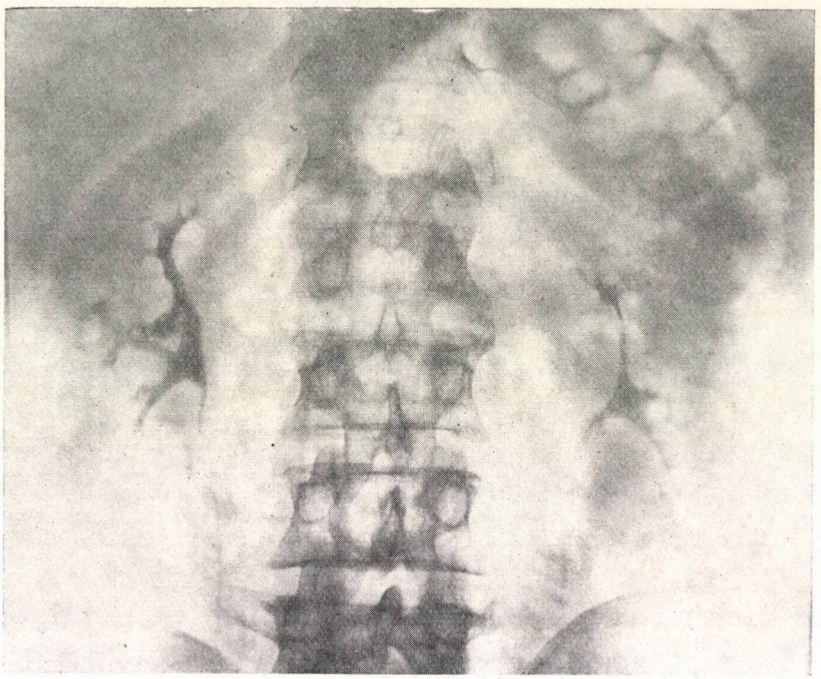

Fig. 1a

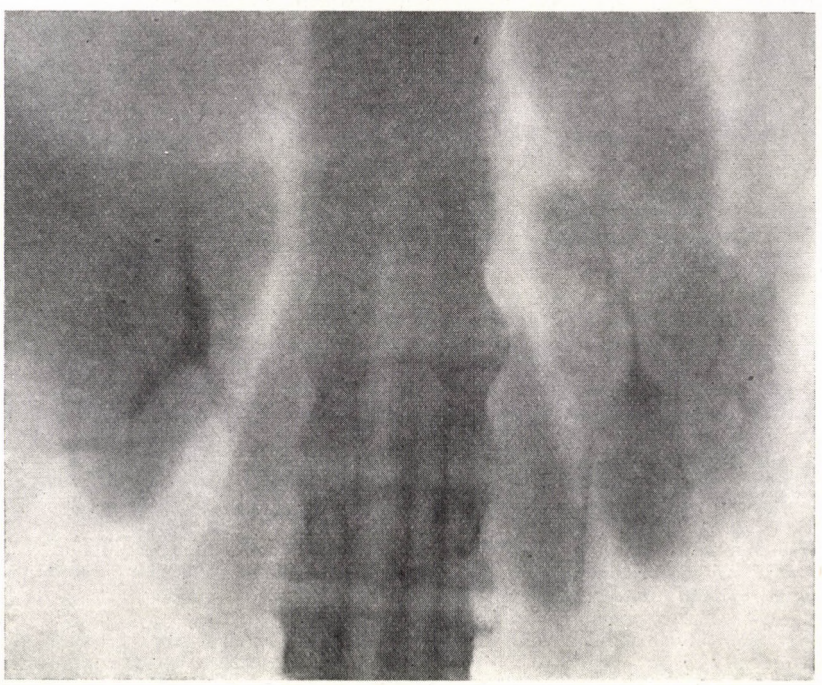

Fig. 1b

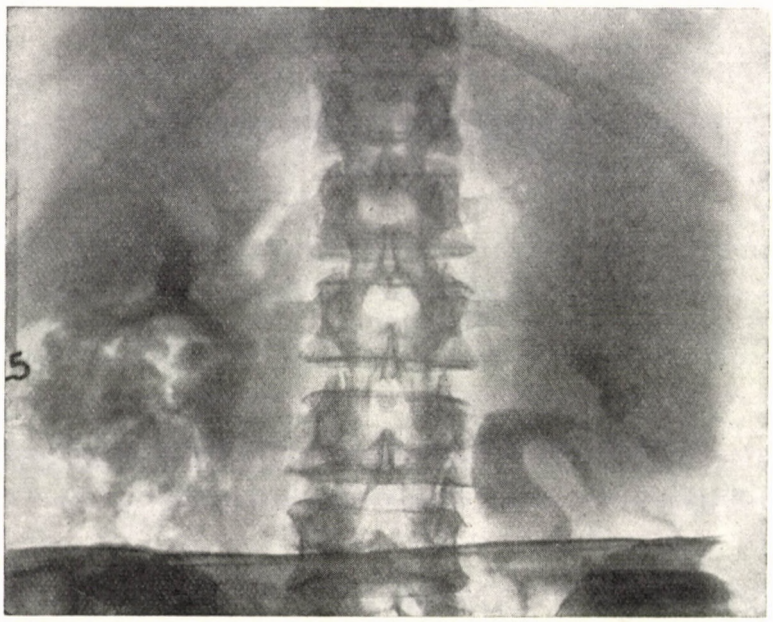

Fig. 2a

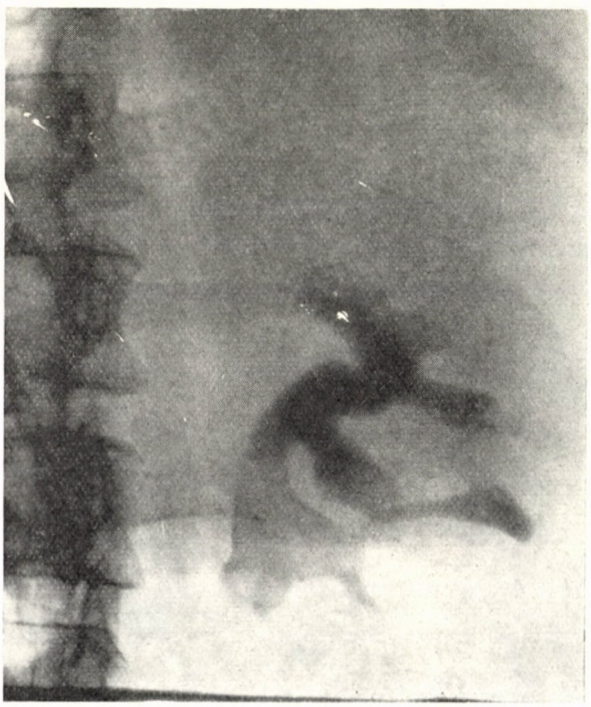

Fig. 2b

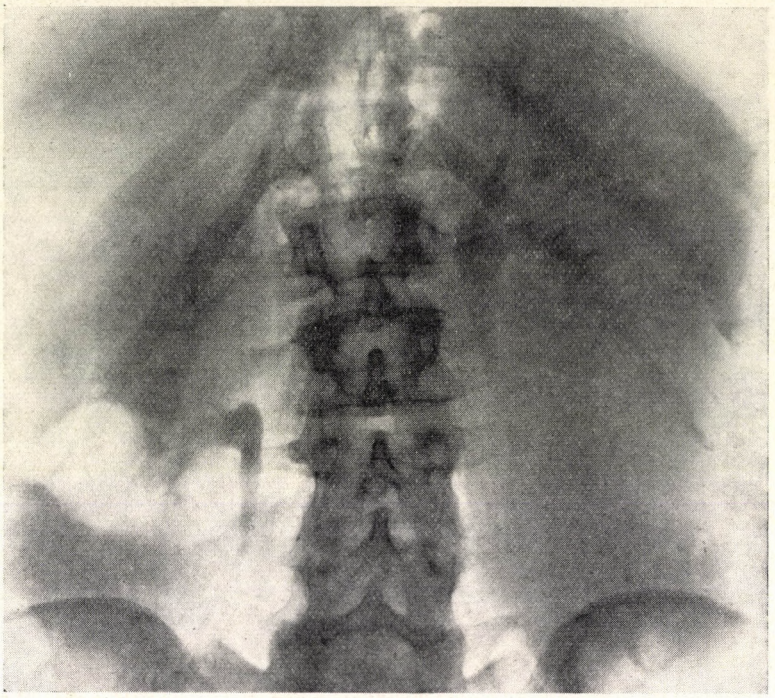

Fig. 3a

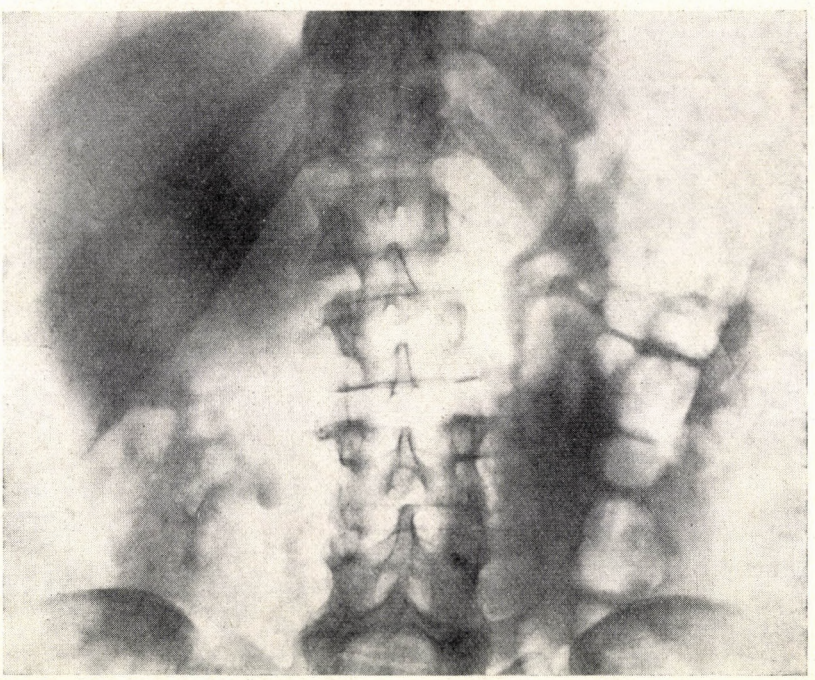

Fig. 3b

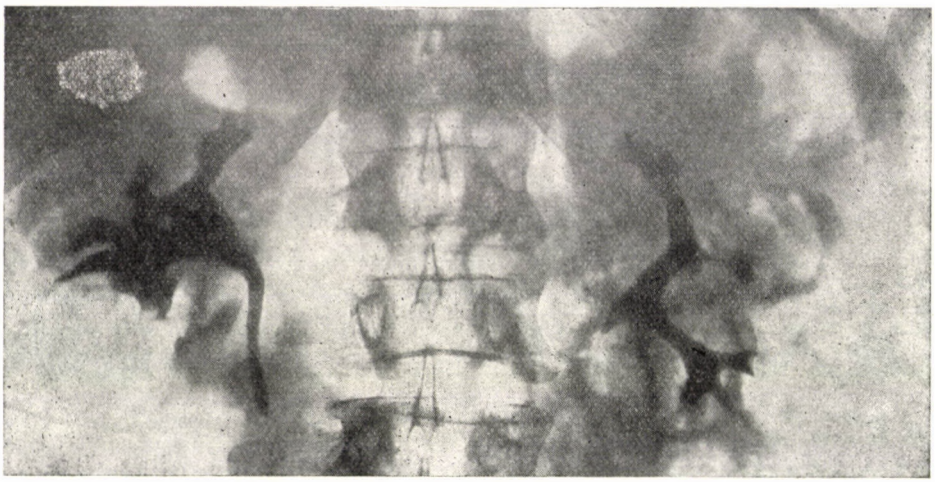

Fig. 4a

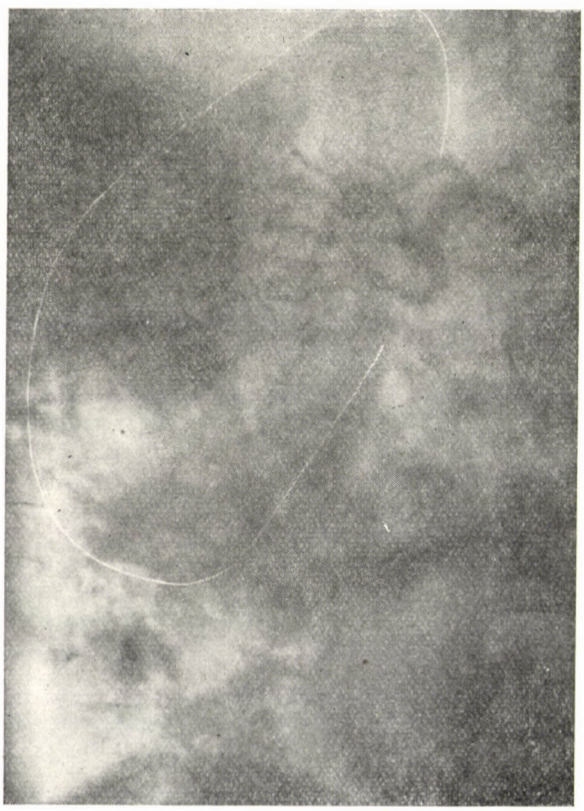

Fig. 4b

increased to an enormous size. For this reason, splenectomy was performed. As shown by the postoperative urogram, the displacement of the left kidney in consequence of splenomegaly had practically disappeared.

Case 3. (Figs 3a, b), S. J., a 47-year-old female. The upper pole of the left kidney is seen at the level of the posterior part of the 10th rib, i.e. 3 cm higher than normally and 2 cm towards the midline. The pyelo-ureteral junction is straightened out. The lower pole of the right kidney reaches down to the line of the iliac crest, being displaced caudally by 4 cm and medially by 2 cm. Its axis is parallel with the spine. The caliceal stalks are at right angles to the renal pelvis.

On the urogram performed one month after splenectomy (3b) the left kidney appears normal in size and of practically normal position, with its upper pole at the lower border of the 11th rib and its axis parallel with the psoas muscle. The position of the right kidney was unchanged.

In two subjects, unilateral cysts of the kidney were found. Since, on the evidence of clinical observations [14, 18] renal cysts belong to the primary processes giving rise to renal polyglobulia, this possibility had to be considered. However, the typical bone marrow picture, with leukocytosis, thrombocythaemia, enlargement of liver and spleen left no doubt about the diagnosis of polycythaemia vera. One of the cases is reported in the following.

Case 4. (Fig. 4a), M. G., a 61-year-old male. The left kidney of normal shape and size is situated 3 cm lower than normally. Cranially and laterally from there is the shadow of the considerably enlarged spleen. The left ureter appears slightly tortuous. The right kidney is of normal position, its lower half appears broader than normally. The lower and middle calices fill poorly. One of the middle calices has a tapering form, as a sign of compression which involves the renal pelvis. The area of compression is sharply outlined upwards and shows rounded contours.

Selective arteriography of the right kidney (Fig. 4b) revealed the intrarenal branches of the renal artery supplying the caudal half of the kidney to curve upward and to enclose a poorly vascularized area of the size of a tennis ball. In later phases there was no arteriographic sign of any functioning renal parenchyma within this area, indicating the presence of a cyst in the right kidney.

Discussion

Renal dystopia demonstrable by X-rays constitutes a primary anomaly or it may be secondary to retroperitoneal tumours, splenomegaly or to tumours of the kidney itself [7, 11, 21]. The X-ray features of left renal displacement by splenomegaly have amply been discussed in the literature [5, 6, 7, 15, 17]. Hepatomegaly as a cause of right renal displacement is also well-known [4]. Displacement and rotation of the kidney involve certain differential diagnostic problems [6, 7, 14, 21] of decisive importance as regards the therapeutic course to be adopted.

FORDE et al. [5] were able to demonstrate left renal displacement by intravenous urography in 25 out of 27 cases of splenomegaly due to various causes, in the first place to diseases of the haematopoietic organs, cirrhosis of the liver and splenic cysts.

DELAMORE et al. [4] found renal displacement in consequence of hepatomegaly or splenomegaly in 21 out of 25 cases of polycythaemia vera. There was no primary renal disease in their series.

The present study, which has been aimed at the X-ray investigation of the kidney in polycythaemia vera, involved 30 subjects; 29 of them had splenomegaly, 27 hepatomegaly. Splenomegaly of grade +++ was present in 9, hepatomegaly of the same grade in 8 cases. Displacement of the left kidney

was demonstrable in 28, that of the right kidney in 17 cases. Splenomegaly as well as hepatomegaly of grade +++ were usually accompanied by combined, i. e. caudo-medial displacement of the kidney associated in most cases with a rotation of the organ. (Owing to methodological considerations, the incidence of a possible displacement in the sagittal direction as observed by WEISS [21] was not investigated in this study.) In the entire series, the extent of renal displacement was closely related to the haematological status expressing the prevailing clinical condition (recurrence or remission) as also to the extent of hepatomegaly or splenomegaly: while the X-ray signs of renal displacement noted during the periods of recurrence showed a definite regression during remissions, those demonstrable at the times of remission were found to have increased in degree in the case of a recurrence. The above observation where all signs of a combined renal displacement disappeared after splenectomy, convincingly illustrates the reversibility of these changes.

Other abnormalities found included pyelectasy in 2 and nephrolithiasis in 3 cases, all with marked renal displacement. There is every reason to consider the renal displacement, or rather the ureteral angulation thus produced, as a predisposing factor of pyelectasy as well as of renal calculosis, the more so as polycythaemia had been present for long years in both cases (8 and 10 years respectively), therefore renal displacement must also have been present for a long time. PINTÉR and FORRAI [15] reported a case of myelosclerosis where the combined displacement of the left kidney due to splenomegaly was associated with renal calculosis.

It was remarkable to find two subjects with unilocular cysts of the kidney. The possibility of renal polycythaemia could, however, be ruled out in both cases on the grounds of the classical features such as splenomegaly, hepatomegaly, typical bone marrow, leukocytosis, and thrombocythaemia.

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COMPLEX CARDIORESPIRATORY INVESTIGATIONS WITH MINIMAL ERGOMETRIC LOAD FOR SCREENING OF PATIENTS IN CARDIAC REHABILITATION*

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A complex oximetric and radiocardiographic method performed in right heart catheterization during ergometric load has been worked out. The $^{133}X_e$-radiocardiogram proved well reproducible and its course during the load was planimetrically proportionate to the dilution curve of the dye injected simultaneously.

In the course of the loading reaction there was no significant difference in cardiac output, stroke volume and arterial oxyhaemoglobin content between a normal and the postinfarction group two years after the acute episode. In the latter group the increase in heart rate and recuperation of the $^{133}X_e$ regional lung clearance were somewhat delayed. The cause of this and of the modest increase in alveolar ventilation might have been due to the postinfarction rehabilitation training.

To facilitate interpretation typical loading diagrams are presented of the haemodynamic hyperreaction of a patient with labile hypertension, of one with compensated chronic cor pulmonale and one with primary cardiomyopathy associated with absolute arrhythmia.

Selection of postinfarction and heart-operated patients for rehabilitation training must occur in the knowledge of their haemodynamic parameters, in addition to the usual ECG, radiologic and spiro-ergometric findings. For this purpose a complex oximetric and radiocardiographic loading technique has been worked out.

Material and method

A so-called normal group of ten middle aged male patients served for determining the normal reaction. They had no valvular defect or cardiomyopathy and were admitted for a temporary increase of blood pressure in the functional stage of hypertension. Their ECG was normal in twelve derivations and the load did not lead to pathological changes. The second group consisted of ten patients who were in good condition two years after having suffered a myocardial infarction; their ECG showed residual repolarisation disturbances which did not change on applying the 50 W bicycle ergometric load in the sitting position.

The equipment consisted of a four channel radiocirculograph, an Atlas Universal Oxymeter, an Atlas-Krupp Cardiognost-Recorder and an electrocardiograph. An analogue computer (Berthold product linear-logarithmic ratemeter) connected to the magnetic data storage system served for putting into logarithmic order and integrating the recorded activity-time curve.

At the start of the experiment, arterial oxyhaemoglobin content, lung-ear circulation time, basal ECG and carotid curve were determined. After right heart catheterization with a thin flexible polyethylene catheter we recorded electromanometrically the pressure in the great veins, in the right auricle and in some cases in the right ventricle. Then at rest two indicator boluses one after the other were injected into the right auricle, then one bolus every one

* This work was supported by the International Atomic Energy Agency.

and a half minute during four minutes ergometric load and in the recuperation period further indicator portions were injected. Loading was performed with a bicycle ergometer in the recumbent position, after the patient has used the ergometer for a short time.

The recumbent position was necessary in view of the radiocardiographic arrangement. The reason for minimalizing the ergometric load to 50 W and for departing from the generally accepted 1 W/kg was to establish a bottom limit, under which a pathological response excluded the patient from the rehabilitation training. At the same time, the 50 W loading level coincided well with one of the loading grades obtained in the sitting position applied by us for the routine classification of postinfarction patients.

The indicator boluses consisted of $^{133}X_e$ dissolved in saline, of methylene blue with an identical concentration of $^{133}X_e$ and, at the conclusion of the experiment, after the heart rate had returned to its basal value, of ^{131}I human serum albumin with an identical concentration of methylene blue. During the rest, the loading and the recuperation periods at least one X_e-methylene blue combination was available for the simultaneous control of the changes measured by radiocardiography and dye dilution. $^{133}X_e$ was chosen in view of its chemically inert nature, in consequence of which it leaves the organism through the lungs at the first recirculation already, so we could measure the activity in periods of one and a half minutes without having to reckon with a residuum. Another advantage of $^{133}X_e$ is that it allows simultaneous washout examinations for estimating the $^{133}X_e$ regional lung clearance. The ^{131}I-albumin radiocardiogram obtained after normalisation of the heart rate, supplied the quantitative basis of the relative $^{133}X_e$ cardiac output data. The rate of change is characterised by the proportion of the planimetred area, as identical activities have been injected.

The ^{131}I-albumin radiocardiographic procedure [1, 2] was based on the principle of selective quantitative radiocardiography introduced by DONATO et al. [1] except that as point of detection the crossing of the pulmonal artery and of the aorta was used. Estimation of the regional alveolar ventilation in the right upper lobe was performed by the $^{133}X_e$ washout method after intravenous injection through a micro-catheter, introduced by HEKSCHER, LARSEN and LASSEN [4].

Methodical demonstration

In the right upper corner of Fig. 1 the activity calibration, in the left upper corner the basal $^{133}X_e$ radiocardiogram and regional clearance curve, in the centre the logarithmical and integrated changes can be seen. In the second, third and fourth lines, the data of the one and a half minute periods from loading until recuperation, while at the bottom line the basal expirational apnoea-curve, the ^{131}I-albumin quantitative radiocardiogram during

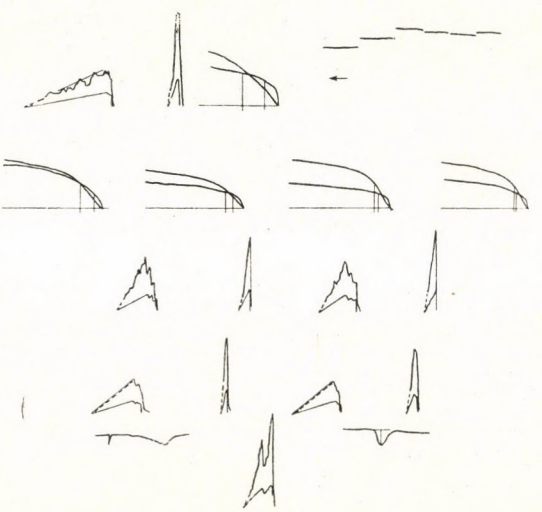

Fig. 1. Complex radiocardiographic and oximetric investigation during load. For details, see text

recuperation and the simultaneous methylene blue dye dilution curve are visible. Flow and cardiac output were estimated on the basis of the first exponential component of the $^{133}X_e$ radiocardiogram. As the activity was introduced through a catheter, the areas of the two basal $^{133}X_e$ radiocardiograms and of the two basal regional clearances were in good agreement.

Results

In the figures, heart rate, stroke volume, the percentual changes of cardiac output, of the regional $^{133}X_e$ clearance, and of oxyhaemoglobin are shown, together with cardiac output at the end of the recuperation period, the regional $^{133}X_e$ clearance in ml/min related to 1 ml of alveolar air, the circulating blood volume and the dye dilution/concentration quotient.

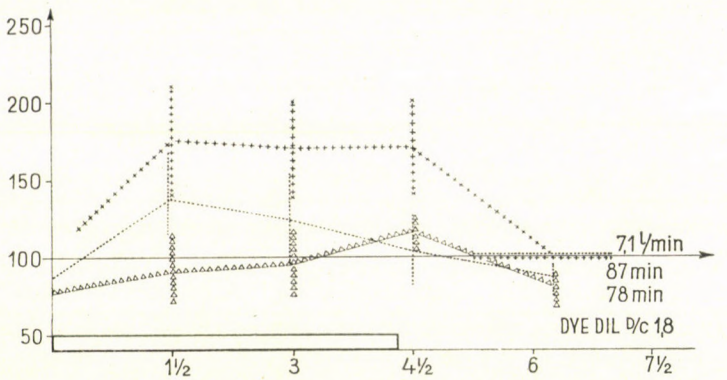

Fig. 2. 50 W ergometry in recumbent position, 4 min. Normal cases, n = 10. Per cent of resting value: cardiac output +++, heart rate (min) ..., stroke volume (ml) △△△△

Fig. 2 shows that in the normal group heart rate and cardiac output reached their maximum in the first one and a half minutes. Cardiac output then remained constant at the 175% level, while the initial heart rate of 140 began to decrease already during the loading; this caused a continuous increase in stroke volume during the whole time of loading. Scattering of the values for the listed parameters was ±20%. Mid-point of the recuperation of all three parameters took place in the 6th minute, at the same time the average dilution/concentration was normal.

In Fig. 3 the course of the regional $^{133}X_e$ clearance is similar to that of cardiac output, only the rate of the change is around 225% and the scatter only 10%. Arterial oxyhaemoglobin saturation starts from a normal level, it decreases after half a minute to return half a minute to or in some cases to somewhat more than its starting value.

In the postinfarction group, heart rate, cardiac output and stroke volume had reached their highest level in the third minute. Cardiac output increased to about 175%, just as in the normal group, the increase in heart rate was

less and of the stroke volume more than of the normal group; the difference in heart rate was considerable. The maximum scatter was about ±20% in all three parameters. Their restitution occurred around the seventh minute, at the same time as did that of the dilution/concentration quotient.

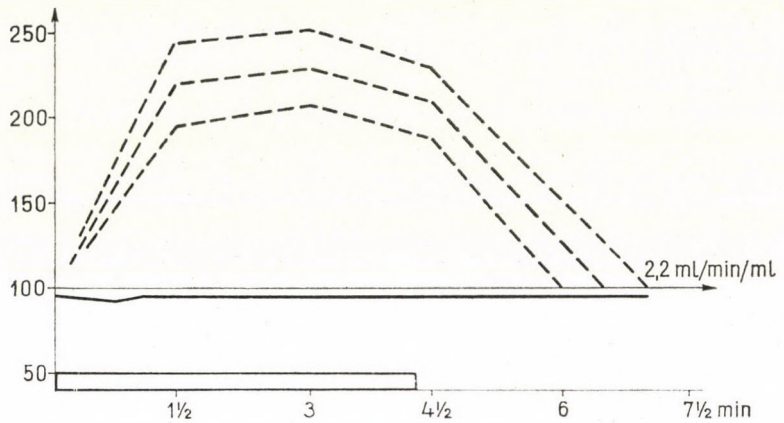

Fig. 3. 50 W ergometry in recumbent position, 4 min. Normal cases, n = 10. Per cents of resting value: regional $^{133}X_e$ clearance — — —, arterial oxyhaemoglobin level ———

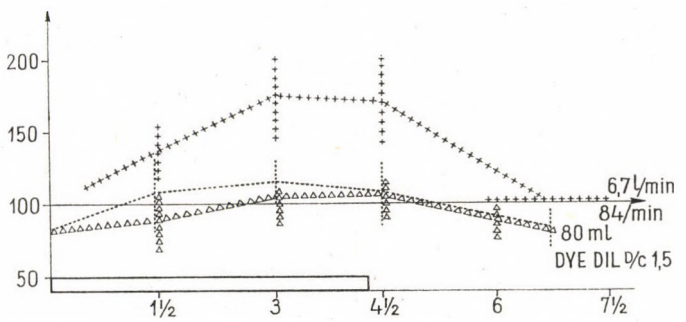

Fig. 4. 50 W ergometry in recumbent position, 4 min. Postinfarction cases after two years. n = 10. Per cent of resting value: cardiac output +++, heart rate (min) ....., stroke volume (ml) ΔΔΔΔ

The cause of this more favourable heart rate reaction, as well as of the more modest increase of alveolar ventilation might have been due to that the postinfarction patients took part in constant training.

In Fig. 5, the course of the clearance curve in the postinfarction group is similar to that of cardiac output; the peak amounts to 180% in the third minute. Arterial oxyhaemoglobin saturation starts from a lower value in the normal group, it begins 30 seconds after loading, but at the end of the 2nd minute it reaches the starting level, where it then remains.

Fig. 6 is the summation of the former figures, offering a comparison of the values for heart rate, cardiac output, stroke volume, regional $^{133}X_e$ clearance and arterial oxyhaemoglobin in normal and postinfarction patients.

Heart rate and cardiac output reached their peak values one and a half minutes later and lasted in the recuperation period half a minute longer in the postinfarction group than in the normal one. Taking the scatter into account, the one and a half minute delay in the normalization of cardiac output

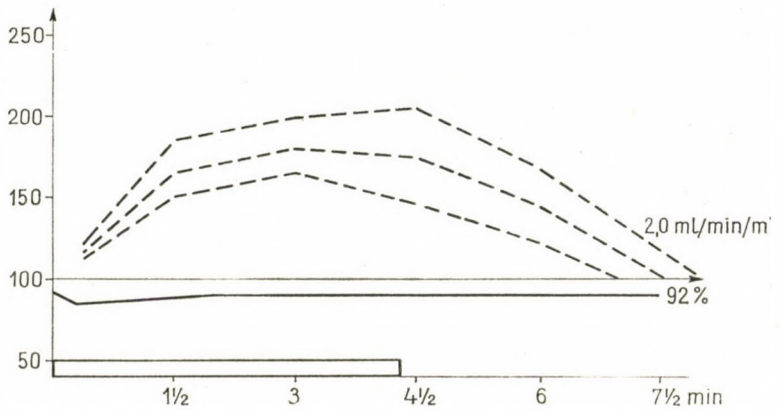

Fig. 5. 50 W ergometry in recumbent position, 4 min. Postinfarction cases after two years, n = 10. Per cents of resting value: regional $^{133}X_e$ clearance — — —, arterial oxyhaemoglobin level ———

approaches statistical significance, whereas the delay in heart rate normalization is significant. It is the $^{133}X_e$ clearance which deviates most in the recuperation. The postinfarction patients starting at a somewhat lower oxygen saturation level, in the first minute of the load display a value somewhat lower than the normal, and the basic level sets in about half a minute later in accordance with the retarded adaptation.

Fig. 7 shows cardiac output and stroke volume in a patient with labile hypertension with a fast increase in heart rate. Similar reactions were observed in the normal group, too, and they were probably slightly increasing the one and half minute mean value in the normal group.

Fig. 8 shows the reaction to loading of a patient with compensated chronic cor pulmonale: a significant increase in heart rate, variable cardiac output and stroke volume pattern, distinct desaturation and a definite increase in alveolar ventilation were typical in this case.

Fig. 9 presents the even more unfavourable reaction of a patient with cardiomyopathy and an auricular fibrillation; there was a great increase in heart rate, a temporary augmentation of cardiac output in the first minute of loading, while stroke volume remained at an approximately constant low level.

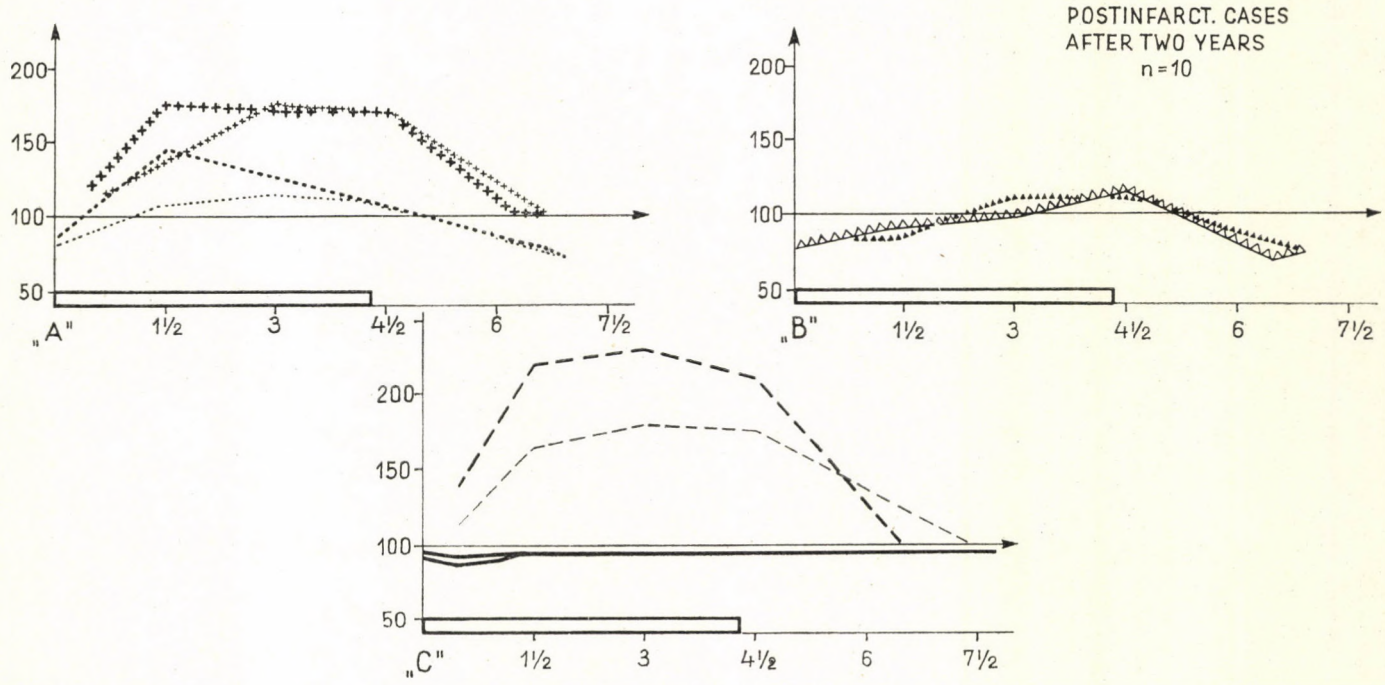

Fig. 6. Summation of Figs. 1 to 5. Mean values. Normal and postinfarction cases after two years. n = 10 each Above left, comparison of cardiac output and heart rate. Above right, comparison of stroke volumes. Below, comparison of regional $^{133}X_e$ clearance and oxyhaemoglobin level

In the fourth minute the patient became dyspnoeic and loading was discontinued. At this time, desaturation had become apparent and cardiac output began to decrease.

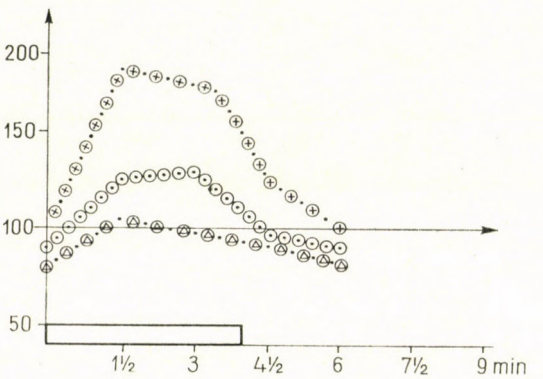

Fig. 7. 50 W ergometric load in recumbent position, 4 min. Labile hypertension. Per cent of resting value: cardiac output +++, heart rate (min) ...., stroke volume (ml) ΔΔΔΔ

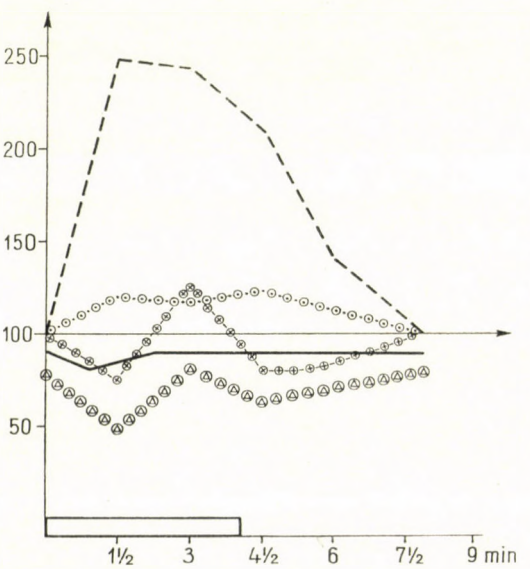

Fig. 8. 50 W ergometry in recumbent position, 4 min. Chronic compensated cor pulmonale. Per cents of resting values: cardiac output +++; regional $^{133}X_e$ clearance — — —; heart rate (min) ....; stroke volume (ml) ΔΔΔΔ; arterial oxyhaemoglobin level ———

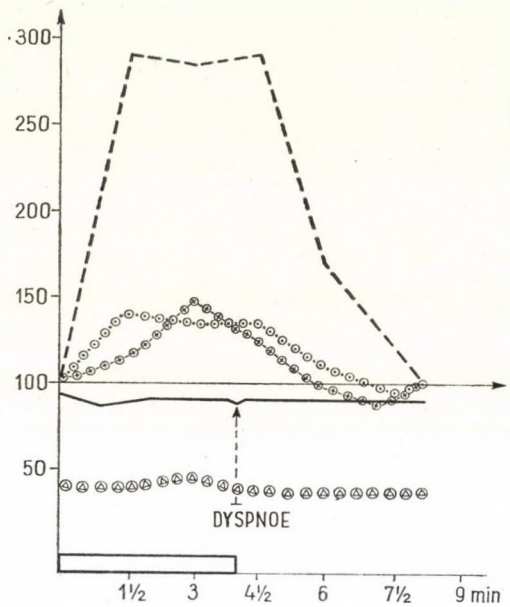

Fig. 9. 50 W ergometry in recumbent position, 4 min. Myocardiopathy with absolute arrhythmia. Per cents of resting values: cardiac output +++; regional $^{133}X_e$ clearance ———; heart rate (min) ....; stroke volume (ml) △△△; arterial oxyhaemoglobin level ———

Discussion

The obtained data show that the haemodynamic reactions of loaded patients who were in a good condition two years after myocardial infarction, did not differ significantly from the group of normal middle-aged men. The initial slower elevation of heart rate and cardiac output in the postinfarction patients does not allow far-reaching quantitative conclusions, since the initial curve of the stroke volume in both investigated groups ran its course at a nearly identical level, which approximately equalized the retarded starting.

Normalization of cardiac output in the normal group set in at the sixth minute while in the postinfarction group it ensued around the seventh minute, with half a minute difference. The difference was even more pronounced in the case of regional $^{133}X_e$ clearance.

The scatter in the results is considerable, but we can hardly expect a better one, taking into account that the baseline values may already show a deviation of nearly 10% and that the course of the reaction depends greatly on the vegetative tone. When evaluating the instantaneous situation, we must reckon with a fairly wide normal zone. At any rate, comparing simultaneously several parameters minimizes the possible errors.

The changes in heart rate and cardiac output observed by us were somewhat higher than those reported [5] for a 50 W bicycle load in sitting position; this might be due to a somewhat different distribution of the cardiac output during the loading in the recumbent position.

Knowing the normal values and the reactions to loading of the cardiac output, stroke volume, arterial oxyhaemoglobin and the heart rate and $^{133}X_e$ clearance, our method might be of help in the earlier selection of postinfarction patients.

Methodical addendum

For measuring one of the detectors was placed over the right ventricle in the fourth intercostal space next to the sternal margin; for the regional clearance we chose the right upper lung zone and detected from the right third intercostal space in the midline of the clavicle. Since we examined the changes in relation to the initial phase, the geometrical difficulties originating from the absorption of soft-gamma rays did not count much.

The $^{133}X_e$ radiocardiogram was considered one from the side of the right heart, but this differentiation was of interest only in the adaptation occurring in the course of the first minute [6]. Later, cardiac output from the two sides of the heart is identical even under the effect of loading.

An Evans-blue series allowing a reliable quantitative determination would have loaded the patient to a much greater extent than the quickly excreted methylene blue had done.

Comparison of the planimetred areas and impulse rates

	In basic conditions		Repeated 2nd injection	
^{133}Xe primary radiocardiogram	10.1±1.2 cm²	302±25 cps	9.7±0.9 cm²	280±26 cps
$^{133}X_e$ regional clearance	18.8±1.5	585±61	18.2±1.6	558±56

Comparison of the planimetred areas of the $^{133}X_e$ primary radiocardiogram and of the methylene blue dye dilution curve

	Start	3 min	At the end of recuperation
$^{133}X_e$ primary radiocardiogram	9.9±1.0 cm²	5.9±0.7 cm²	9.6±1.0 cm²
Methylene blue dye dilution curve	6.2±0.7	3.6±0.4	6.0±0.7
	n = 20	p = 0.05	

The radiation load was minimized as each of the 200 μC_i $^{133}X_e$-boluses left the organism within one and a half minute and the terminal ^{131}I-albumin activity for cardiac output calibration was 20 μC_i ^{131}I.

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EFFECT OF VITAMIN K_3 ON HUMAN AND RAT RENOCORTICAL SUCCINIC DEHYDROGENASE ACTIVITY

By

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Succinic dehydrogenase activity was demonstrated histochemically in isolated rat glomeruli. The activity of the glomerular enzyme was estimated quantitatively under the effect of various substances affecting the oxidoreductive processes of the cell. The sites of interference of two tetrazolium salts (nitro-BT, TPC), and of vitamin K_3, were located within the oxidation cycle of succinic acid. Human nephritic kidneys showed a reduced succinic dehydrogenase activity which, unlike in normal or nephrotic human kidneys, was hardly enhanced by vitamin K_3, particularly in the glomeruli. A reduction in the activity of the Krebs-cycle enzymes indicates that the metabolic processes of the nephritic kidney are profoundly affected.

While, on the evidence of histochemical methods, the maximum amounts of succinic dehydrogenase (SDH) in both the human and the rat kidney are contained in the ascending limb of Henle's loop and in the proximal and distal convoluted tubules (WACHSTEIN 1955), these procedures, even in combination with electron microscopy, fail to detect any SDH activity in the glomeruli (KERPEL and HAJÓS 1968). However, NOWINSKI and PIGON (1967) showed the presence of SDH in isolated rat glomeruli by micromanometric measurement of the increase in O_2-uptake induced by succinate. By the elimination of the tubules with their high SDH content which masks the slight glomerular activity, the isolation technique makes the demonstration and estimation of the glomerular enzyme not only accessible to micromanometry but also to spectrophotometry used in combination with biochemical or even with routine histochemical procedures (MAROSVÁRI and TANKA 1969), and by means of tetrazolium salts SDH activity can be as readily estimated as in the renal cortex (KUN and ABOOD 1949).

The quinones contained in the kidney play a significant part in the oxidation of succinate (SLATER, 1961). Thus, ubiquinone enhances considerably the SDH activity measured by tetrazolium salts. Vitamin K_3 has a similar influence (WATTENBERG et al., 1960), an effect which may be connected with a still unidentified quinone ("Q factor") similar in structure to vitamin K_3, which beside ubiquinone is presumably involved in the oxidation of succinate (DE HAAN and KRAAYENHOF, 1970).

In haematuric membraneous nephritis a reduced SDH activity has been noted (MAROSVÁRI et al., 1971). The reduction might be connected with some

alteration in the renal quinones in nephritis. Since renal ubiquinone content remains normal in nephritis (NERVYN and MORTON, 1959), we have studied the possible influence of other quinones such as the "Q-factor" or/and of vitamin K_3 on SDH activity in normal as well as in glomerulonephritic human kidneys.

Material and methods

For the human kidney enzyme studies, a 2% homogenate was prepared from the cortical biopsy material with Ringer's solution in a Potter homogenizer. For the estimation of SDH activity the method of KUN and ABOOD (1949) was employed partly in its original form with 0.1% (2,3,5-triphenyl tetrazolium chloride, TPC) partly in our modification by the use of 0.1% (3,3'-dimethoxy-4,4'-diphenylene)-bis-2-p-nitrophenyl-tetrazolium chloride, [nitro-BT]). For human material only nitro-BT was used as hydrogen acceptor: from the renal cortex of the rat a 20% homogenate was prepared with Ringer's solution and TPC or nitro-BT were used as hydrogen acceptors.

For isolating the glomeruli from the renal cortex the method of FONG and DRUMMOND (1969) was used in our modification (MAROSVÁRI 1970).

Histochemical demonstration of SDH was carried out by the method of NACHLAS et al. (1957) parallel with the quantitative estimation of the enzyme activity.

For the isolation of liver mitochondria the method of SCHNEIDER (1948) was used.

The protein content of the homogenates, glomerular suspensions and mitochondria was measured by the micro-Kjeldahl technique.

Results

In rat glomeruli SDH activity became histochemically demonstrable after an incubation period of 60 to 90 minutes (Fig. 1). In the presence of 600 μg/ml of vitamin K_3 marked activity was demonstrable as early as in 15 minutes (Fig. 2). As measured with nitro-BT, this glomerular SDH activity represented approximately one tenth of the activity of the whole cortical homogenate (Table I); this is why the slight glomerular SDH activity remains undetected by the conventional methods unless the glomeruli are separated from the tubules.

In human kidney sections, tubular SDH activity was clearly discernible after 5 minutes incubation. In the cortex of a human nephrotic kidney slight glomerular activity was demonstrable at 25 minutes (Fig. 3). In the nephrotic kidney, tubular as well as glomerular SDH activity increased significantly in the presence of 600 μg/ml of vitamin K_3 (Fig. 4).

On the other hand, in human nephritis the renal glomerulus failed to display any detectable SDH activity after 25 minutes incubation (Fig. 5), nor did vitamin K_3 increase the enzyme activity to any significant extent (Fig. 6).

In order to clarify the nature of SDH activation by vitamin K_3, we have studied some other substances affecting the oxido-reductive cellular processes for their effect on the SDH-tetrazolium system (Tables I and II). It can be

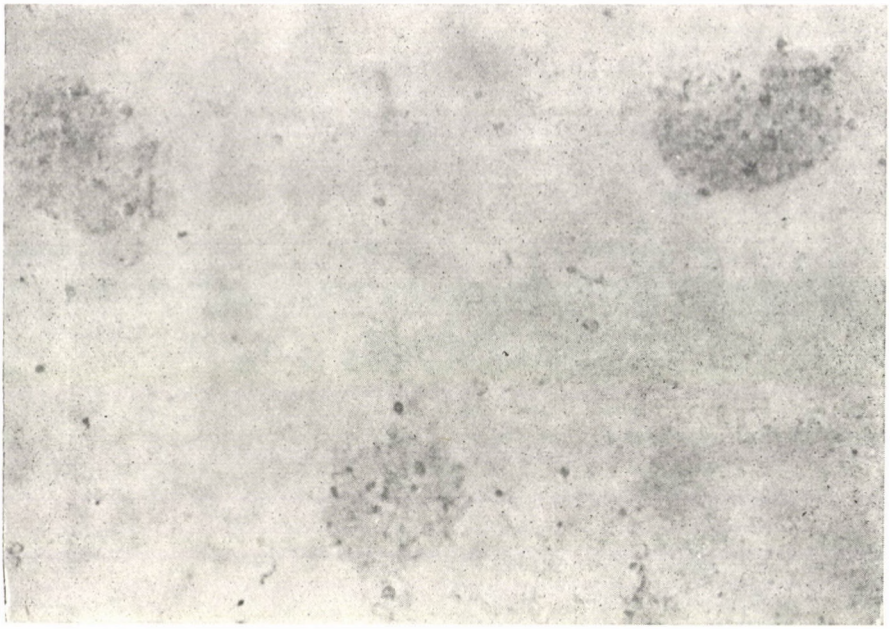


Fig. 1. SDH activity of glomeruli isolated from rat kidney in the absence of vitamin K_3 ($\times 60$)

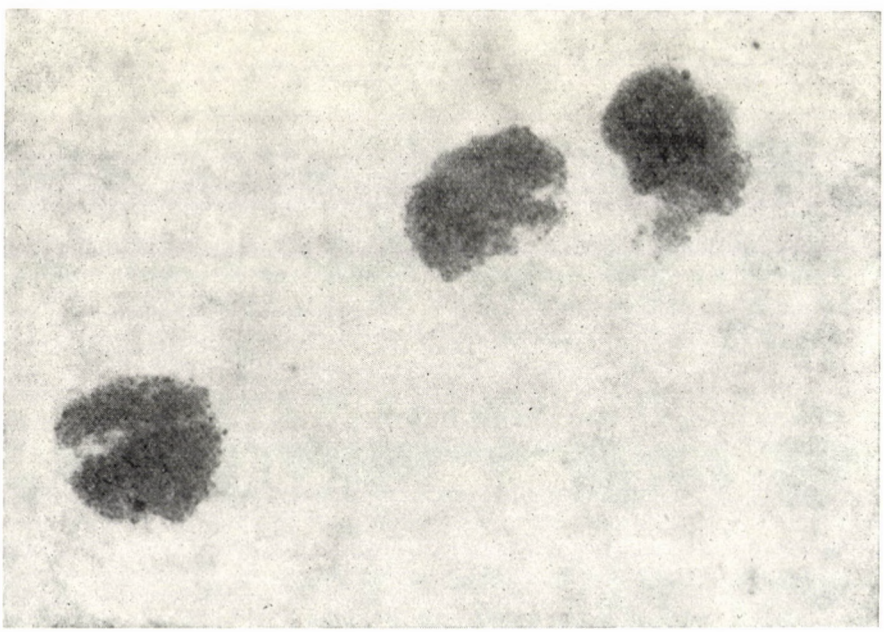


Fig. 2. SDH activity of glomeruli isolated from rat kidney in the presence of vitamin K_3 ($\times 60$)

seen that SDH activity measured by means of nitro-BT, while being more or less inhibited by vitamin K_3 at low concentration, is considerably enhanced at higher concentration. On the other hand, when measured with TPC, vitamin K_3 was inhibiting SDH activity the more the higher its concentration. Potas-

Table I

*Succinic dehydrogenase activity in rat renal cortex**
(10 experiments)

Homogenate with NBT	40±10
+Vitamin K_3 16 μg/ml	26±12
+Vitamin 600 μg/ml	68±14
+KCN 3 μg/ml	64±10
+KCN 110 μg/ml	70±12
+Methylene blue 6 μg/ml	33± 5
Methylene blue 6 μg/ml +Vitamin K_3 600 μg/ml	38± 8
Glomerulus with NBT	3.8±1.5
+Vitamin K_3 600 μg/ml	7.9±2.4

*SDH unit: 1 U = μg formazan/mg protein/10 minutes

sium cyanide had a similar effect in that it enhanced SDH activity when nitro-BT was used and inhibited it when TPC was employed. Methylene blue had no influence by itself on SDH activity and abolished the inhibitory effect of vitamin K_3 in the TPC system. On the other hand, in the nitro-BT system, methylene blue was slightly inhibitory even in itself and even more so on the potentiating effect of vitamin K_3.

Vitamin K_3 affected SDH activity of liver homogenates and even of isolated mitochondria in the same manner as that of renocortical homogenates (Table III). This would suggest that the effect of vitamin K_3 on SDH activity is not organ specific and that it seems to be connected with mitochondrial oxido-reductive processes.

The use of nitro-BT makes the activation of glomerular SDH under the effect of vitamin K_3 clearly demonstrable. TPC does not lend itself to the demonstration of SDH activity under similar experimental conditions since it gives 15 times lower figures than does nitro-BT which, in view of the fact that the activity of the glomeruli represents one tenth of that of the cortex, would be clearly insufficient (Tables I, II).

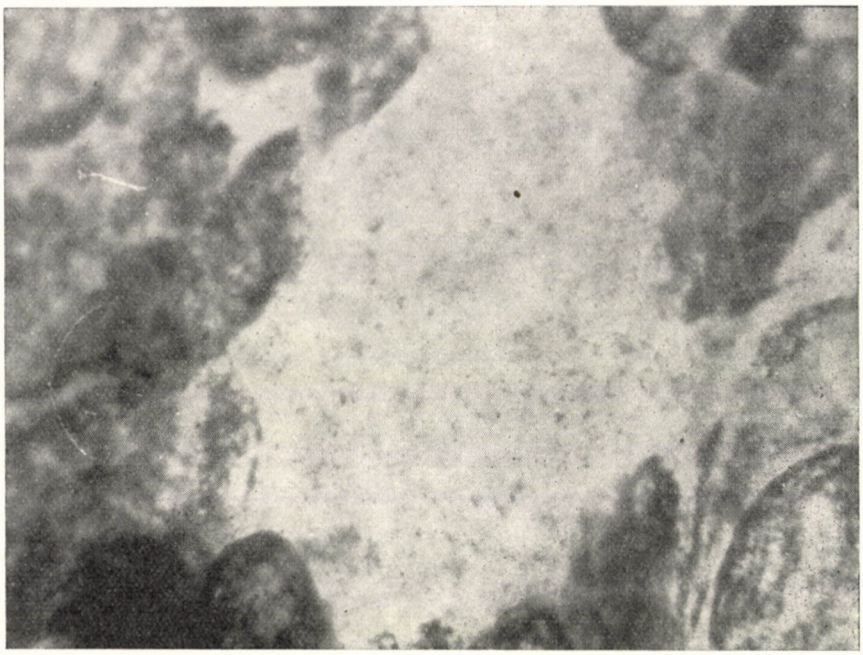


Fig. 3. SDH activity of a glomerulus (centre) and of tubuli from nephrotic human kidney, in the absence of vitamin K_3 ($\times 250$)

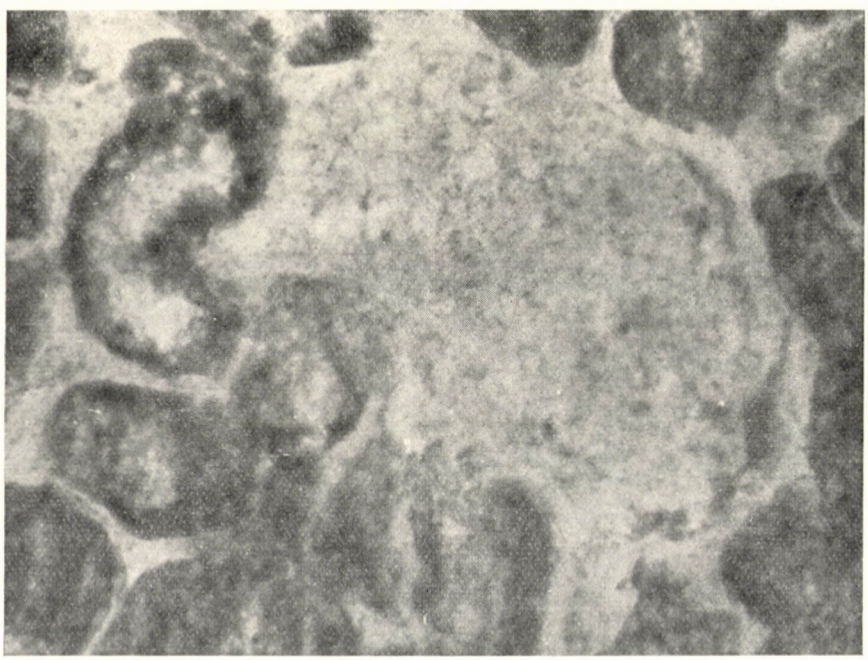


Fig. 4. SDH activity of a glomerulus (centre) and of tubuli from nephrotic human kidney, in the presence of vitamin K_3 ($\times 250$)

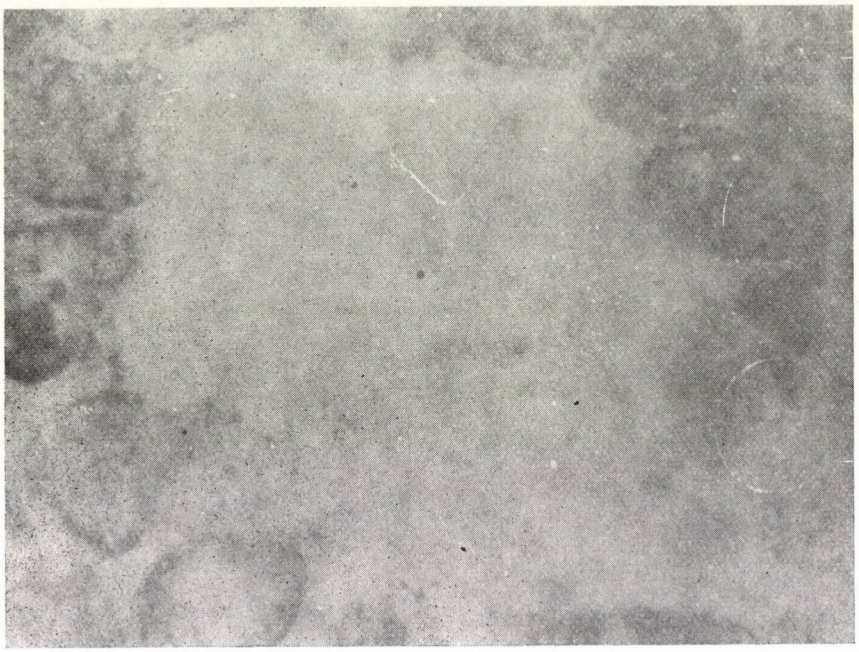


Fig. 5. SDH activity of a glomerulus (centre) and of tubuli from nephritic human kidney, in the absence of vitamin K_3 ($\times 250$)

Table II

Succinic dehydrogenase activity in rat renal cortex
(10 experiments)

Homogenate with TPC	2.5±1.4
+Vitamin K_3 16 μg/ml	1.4±0.6
+Vitamin 600 μg/ml	0.8±0.5
+KCN 3 μg/ml	1.1±0.4
+KCN 110 μg/ml	0.8±0.6
+Methylene blue 6 μg/ml	2.6±1.1
+Methylene blue 6 μg/ml +Vitamin K_3 600 μg/ml	3.1±1.2
Glomerulus with TPC	Ø

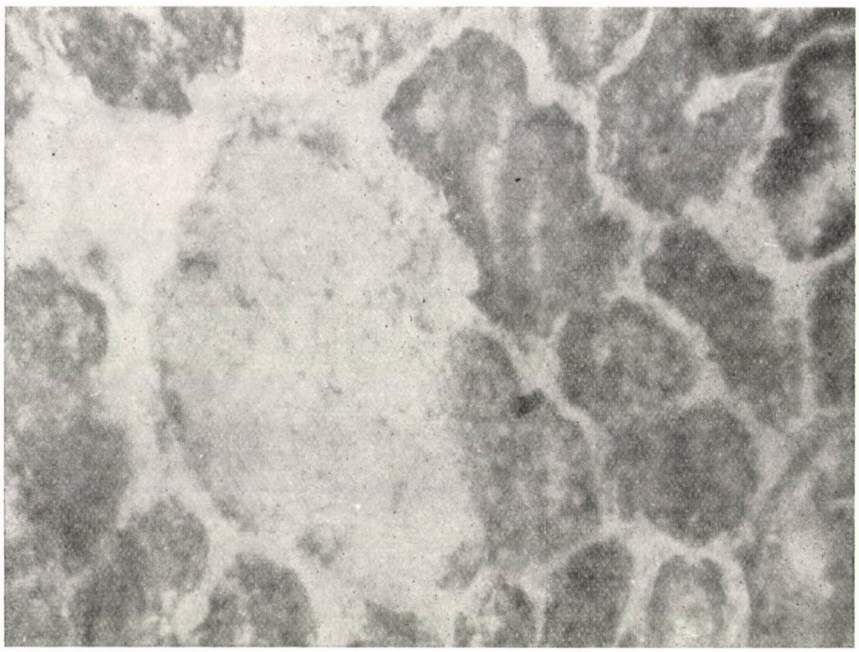


Fig. 6. SDH activity of a glomerulus (centre) and of tubuli from nephritic human kidney in the presence of vitamin K_3 ($\times 250$)

Table III

Liver mitochondria	SDH activity*
+Vitamin K_3 600 μg/ml with TPC	8.0
with TPC	1.6
+Vitamin K_3 600 μg/ml with NBC	120.0
with NBT	152.0

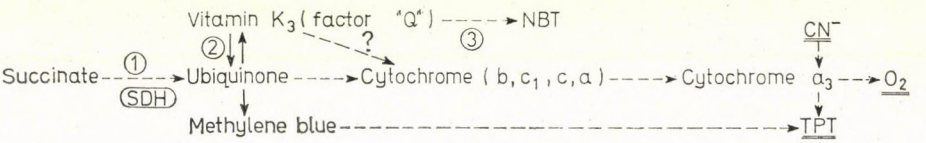
*SDH unit, see Table I

Discussion

The metabolism and enzymology of the renal cortex, in particular of the glomerular apparatus, have been attracting increasing interest. FONG and DRUMMOND (1969) noted an enhanced oxidation of glucose and of palmitic acid in rat glomeruli in the early stages of nephrotoxic nephritis. It was likewise in nephrotoxic nephritis that DUBACH and RECANT (1962) studied the activity of the enzymes involved in the hexose-monophosphate and Emb-

den—Mayerhof shunts and demonstrated an increased G6P-dehydrogenase activity before the manifestation of proteinuria. The question how another essential metabolic pathway, the Krebs-cycle, is affected by nephritis has received little attention. MAROSVÁRI et al. (1971), in biopsy material of human nephritis, found a reduced cortical activity of SDH and of isocitric-dehydrogenase (ICDH). SDH activity in the glomeruli was also weaker and less responsive to vitamin K_3 than under normal conditions. The normal ubiquinone content of the nephritic kidney on the one hand and the failure of vitamin K_3

Table IV



to restore SDH activity on the other, suggest that the reduction in SDH activity may be due to the qualitative or quantitative alterations of the SDH molecule itself rather than to factors interfering at some point with the mitochondrial respiratory cycle, a supposition which agrees well with the presence of hydrogen acceptor links between nitro-BT and vitamin K_3 which have been investigated by KRAAYENHOF and DIEGENBACH (1970). The diagram of this system with respect to the positions of SDH—nitro-BT—vitamin K_3 and TPC is seen in Table IV from which it emerges that if substances taking effect at points 3 and 2 (ubiquinone, factor-Q) are available in adequate amounts, then the disorder responsible for the reduction in the activity of the SDH—nitro-BT system must be connected with disturbances arising at point 1, i.e. on the SDH molecule itself. Clarification of this issue requires further investigations.

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SUBTHALAMOTOMY IN PARKINSON'S DISEASE

ANALYSIS OF RESPONSES TO ELECTROSTIMULATION

By

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The subthalamic target was decided by a new X-ray method with lesion-effect analysis of previous combined thalamo-subthalamic lesions. Evaluating the various stereotactic interventions in the pallidum, in the v.o. part of the VL thalamus and subthalamus, the last one proved to be the most efficient even with a small lesion. Electrostimulation performed for functionally controlling the position of the electrodes resulted in responses and their combinations from 135 points mainly in the v.o. part of the VL thalamus and in the subthalamus. Evaluation of the responses supplied data which seemed to confirm the interactions supposed to exist in extrapyramidal reverberating circles. From the detailed analysis it has been concluded that the v.o. part of the VL thalamus, the n.Sth. and the subthalamic pathways, such as Z.i., and Ra.prl., participate in the elaboration and automatization of some physiologic and pathologic movement-patterns as parts of the extrapyramidal system which is neither a motor nor a sensory system, but a system elaborating and automatizing the conditions of automatic and voluntary movements.

I.

After the era of pallidotomy and thalamotomy and of the different combined lesions, extrapyramidal stereotactic surgery has reached the subthalamus at the beginning of the sixties [1, 3, 10—13].

Some considerations led by the aim of improving the results and of minimizing the size of the lesions have made us to search for the target in the pathway systems instead of the nuclear structures. We found our subthalamic target by analyzing the effect of lesions induced by combined VL thalamic-subthalamic interventions. Evaluating the lesions effective on tremor and tonus, two regions of lesion-concentration were found (Fig. 1). The first was in the VL nucleus, in the territory of v.o.a., v.o.p. and v.i.m. — similar to the target of Hassler — and the second in the subthalamus, in the border region of Ra.prl., H_2 and Z.i. — similar to the target of Mundinger. This subthalamic site seemed to be effective for stopping the tremor and to decrease the tonus even by means of a small lesion. Depending on the size of the ventricles, this subthalamic target structure is situated 10—13 mm laterally from the midline, i.e. 6—7 mm from the wall of the third ventricle, in the plane parallel with the midline and touching the external wall of the lateral ventricle at its infero-lateral corner. In the sagittal plane the target is localized in the first sub-intercommissural and in the second post-midcommissural rectangle, in our rectangular system utilized for aiming and identifying the operative events.

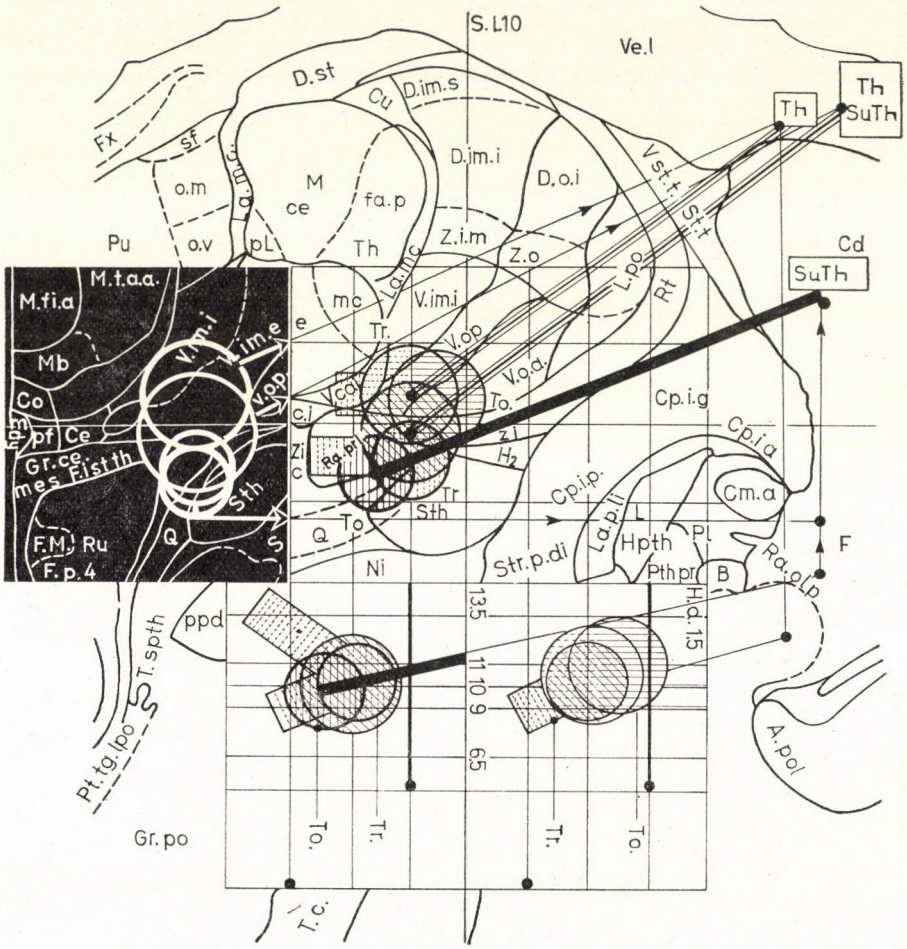


Fig. 1. Location of the effective thalamic (Th), subthalamic (SuTh) and thalamo-subthalamic (Th, SuTh) lesions in sagittal (S.1.10), frontal (F.p.4.) and horizontal (H.d. and H.v.1.5) planes

Analysis of the results showed a somatotopic effect mainly in the subthalamus (Fig. 2), where the hand has a greater representation than have the head, the leg and the body. On the basis of these observations, in the case of a dominance of head, tongue or lip tremor, beside the original target an additional medio-dorsal lesion, or in the case of a leg-tremor dominance, a latero-dorsal one may be placed. The dorsal part of the lesion mainly in-

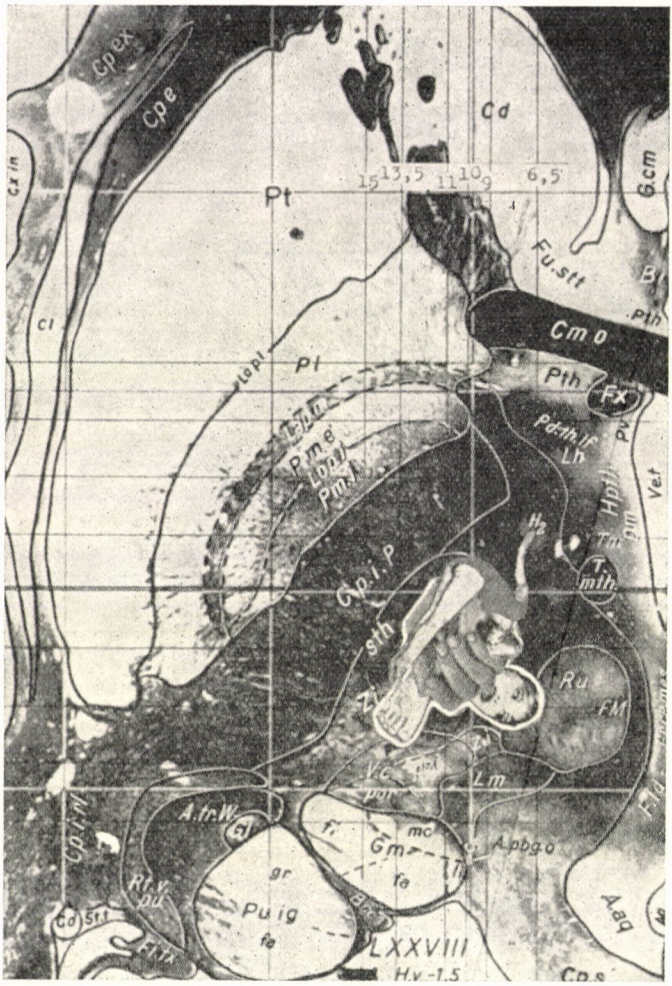


Fig. 2. Somatotopic effect of lesions affecting tremor and tonicity (according to the H.v. 1.5 mm plane of Schaltenbrand-Bailey's atlas). The most significant representation is shown by the lesions affecting the hand. Medially from them towards the Ruber can be seen the representation of the lesions affecting the head, laterally towards the Nigra those affecting the foot, while anteriorly from them is the representation of the lesions affecting the hemi-body, as a whole

fluences the tremor, its ventral part mainly the tonus. According to our experience, a lesion of about 40—60 mm^3 is sufficient to arrest the contralateral tremor and decrease the tonus when aiming is exact, i.e. when the introduction of the electrode in the target already stops the tremor permanently and when low voltage low frequency ES increases the tremor, while high voltage and high frequency ES stops it [4—9].

We have performed 63 stereotactic operations on 55 patients, in 6 cases bilaterally and in 2 cases repeatedly on the same side. In 3 cases surgery was

Table I

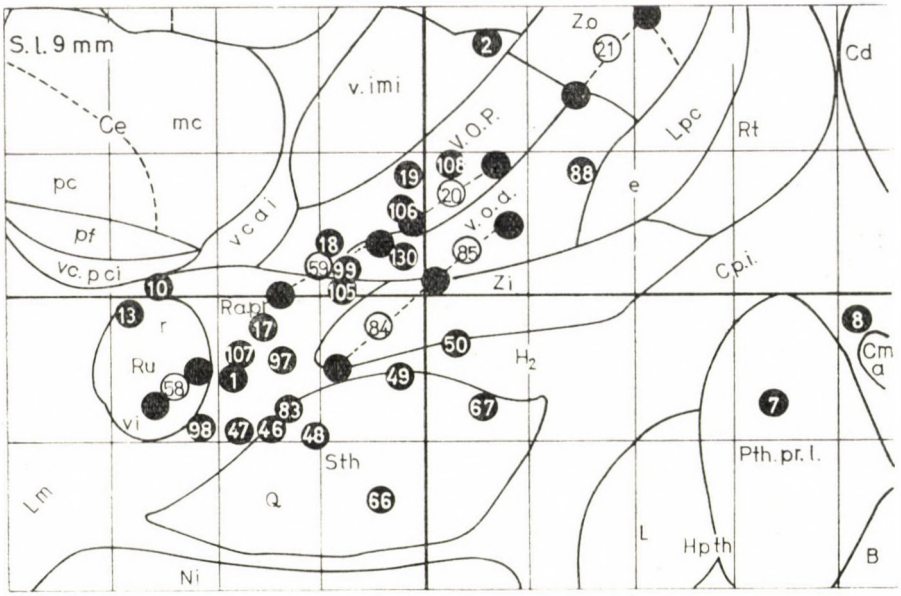
Location of lesion	Number of cases	Number of operations	Lasting (1/2—8 years) good result	Side-effect		Death
				transitional	lasting	
Pallidum m.	5 (2)	5	—	1	1	1 2 months
VL nucleus (v.o.)	3 (1)	3	1	1		1 1/2 hour
VL nucleus and subthalamus	27 (2)	28	22	6	2	3 1/2—1 1/2 years
Subthalamus (Ra. prl., Z.i.)	26 (1)	27	25	4		
Total	61 (—6) 55	63	48	12	3	5

Numbers in brackets denote cases in which the operation performed on the other side was carried out in another structure.

performed in different structures, in 3 cases in the same structures. The results, depending on the sites of the lesions, were well demonstrable (duration of follow up, 1/2—8 years) (Table I). Our results in a few cases of pallidotomy and thalamotomy were poor, but the overall results in many more cases with combined VL thalamic and subthalamic and with isolated subthalamic lesions were encouraging. Both forms of lesions were performed in more than 20 cases, allowing for a reliable comparison of the results. Their evaluation demonstrates that the greatest part of the subthalamotomized patients belonged to the successful group and it was in this group that the fewest postoperational side-effects were noted. Results of bilateral interventions were excellent in 2 cases (a combined VL thalamo-subthalamic and subthalamic lesion and a bilateral subthalamic lesion) and good in one case (bilateral combined VL thalamo-subthalamotomy). In 3 cases (different combined lesions) the results was unsatisfactory. Thus on the basis of our experience, subthalamotomy seems to be the method of choice for the stereotactic treatment of Parkinsons's disease.

II.

For a functional control of the placement and localization of the electrodes electro-stimulation (ES) was applied, with 1—10 v, 10—100 c/s, 1—5 msec square waves and 1—10 sec duration. The points of the ES were registered and summarized in different — 9, 10, 11, 13, 5, 16 mm — sagittal planes (Figs 3 a—f) according to the stereotactic atlas of Schaltenbrand and Bailey.


S. l. 9 mm
Ce
mc
pc
pf
vc. p ci
vc a i
v. imi
V.O.P.
v. o. a.
Zo
Cd
Lpc
Rt
e
Zi
C.p.i.
Ra.pl
r
Ru
vi
H2
Cm
a
Pth. pr. l.
Sth
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Q
Ni
L
Hp th
B

Fig. 3a

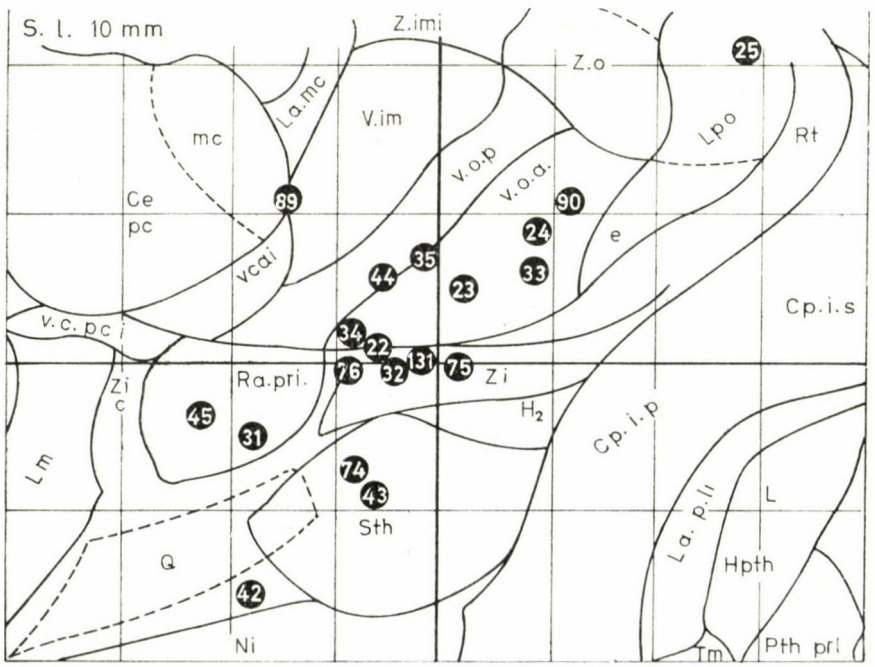
S. l. 10 mm
Z.imi
Z.o
La.mc
V.im
mc
Ce
pc
vcai
v. o. p
v. o. a.
Lpo
Rt
e
Cp. i. s
v. c. pc i
Zi
c
Ra.pri.
Zi
H2
Cp. i. p
La. p. li
L
Hpth
Lm
Sth
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Fig. 3b

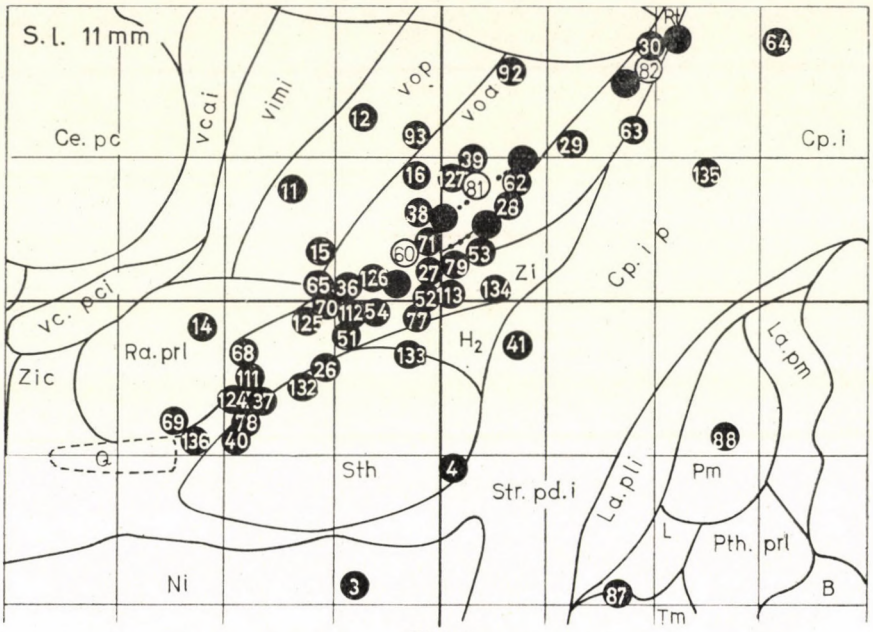
S. l. 11 mm
Ce. pc
vcai
vimi
vop
voa
Rt
Cp. i
Cp. i p
Zi
vc. pci
Ra. prl
Zic
H2
La. pm
Sth
Str. pd. i
La. pli
Pm
L
Pth. prl
B
Ni
Tm
Q

Fig. 3c

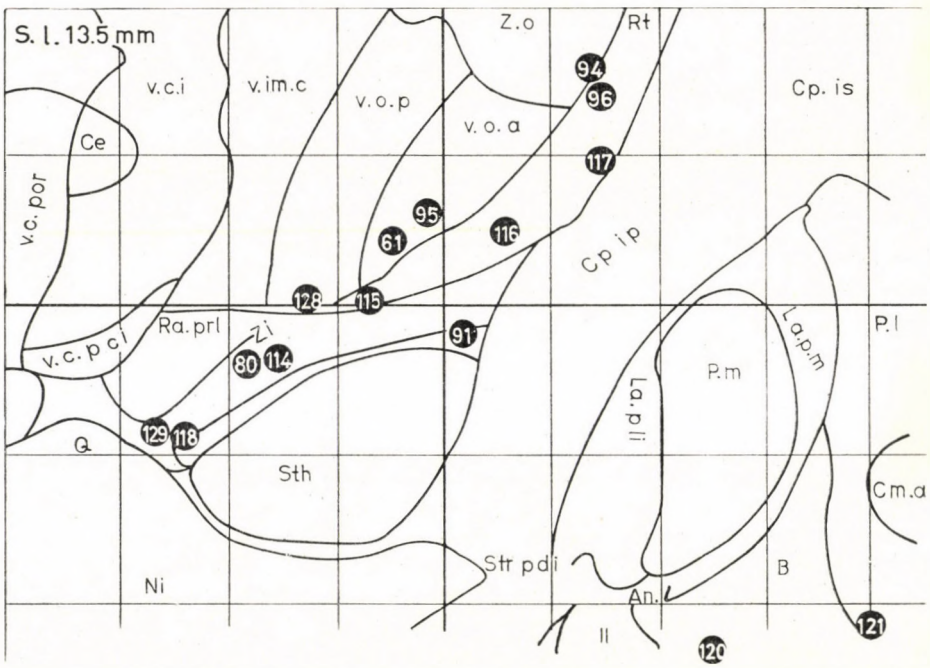
S. l. 13.5 mm
Z. o
Rt
v. c. i
v. im. c
v. o. p
v. o. a
Cp. is
Ce
v. c. por
Cp ip
Ra. prl
Zi
v. c. p c i
P. l
P. m
La. pm
La. pli
Q
Sth
Cm. a
Str pd i
B
Ni
An.
II

Fig. 3d

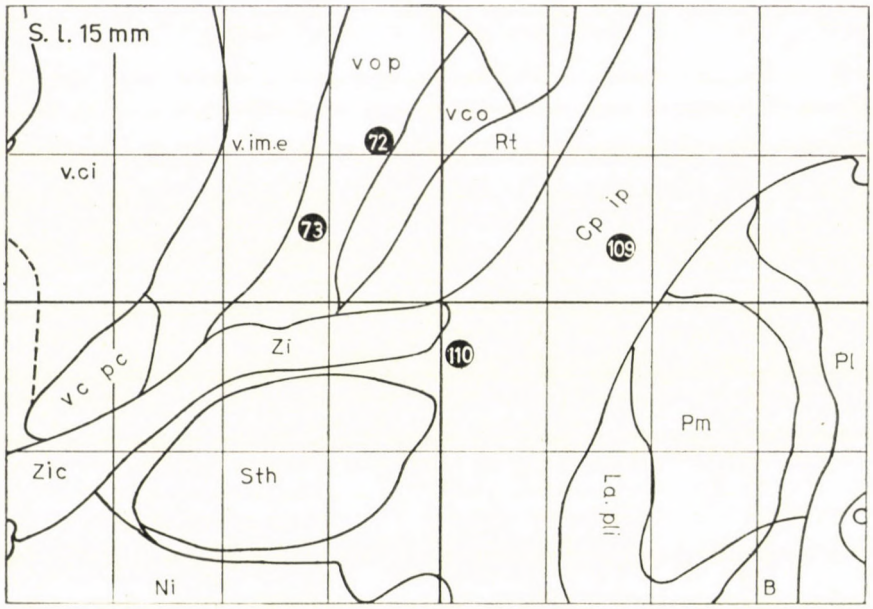

Fig 3e

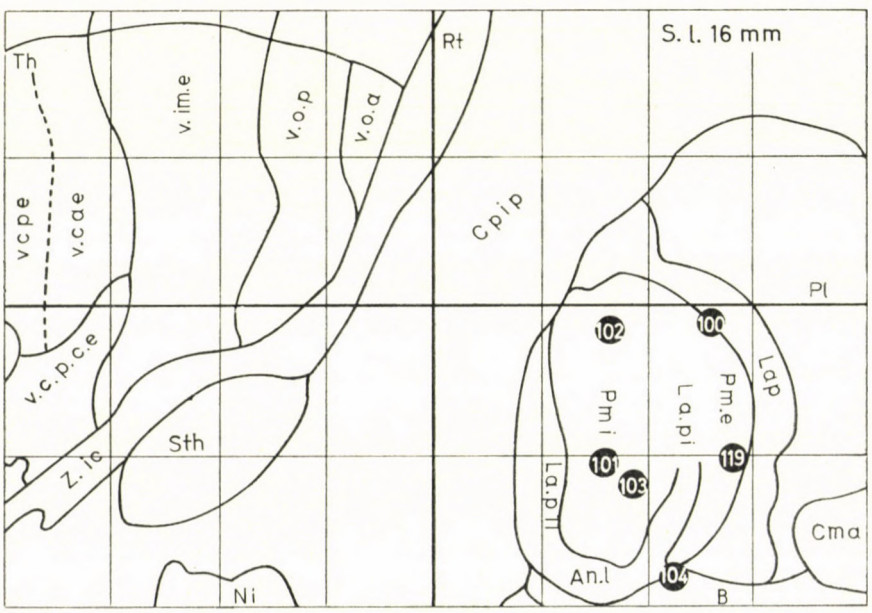

Fig. 3f

Figs 3a—f. Points of ES in sagittal planes. a) 9 mm, b) 10 mm, c) 11 mm, d) 13.5 mm, e) 15 mm, f) 16 mm, from the midsagittal plane

Several stimulations were performed at every point with different parameters. They resulted in different responses and combinations, but the responses were similar in the different extrapyramidal subcortical structures. Careful analysis revealed some remarkable peculiarities. In 135 points — in the VL thalamus; in v.o.a., v.o.p. and in the n.Sth., Z.i., Ra.prl., Ru., P. etc. — about 800 stimulations resulted in more than 1000 responses (Fig. 4).

The responses were grouped as tremor-influencing (activating, inhibiting, altering), speech-influencing responses, sensations, somato, psychomotor

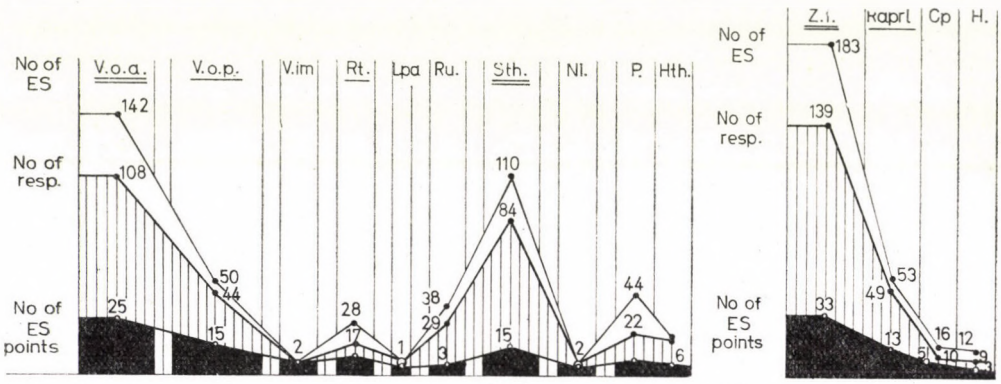

Fig. 4. Bottom: number of points of ES in different structures. Middle: number of responses. Above: number of ES

and automatic manifestations. For detailed analysis, the tremor-influencing responses, sensations and somatomotor responses were subgrouped according to their somatotopic manifestations (head, hand, leg, body), their characteristics, and according to the type of sensation and movement. The purpose of this analysis was an evaluation of the responses and their combinations depending on the threshold stimulus, the stimulation parameters, and on the site of the stimulation. To simplify evaluation, we set up six average parameter categories, which could be done without falsifying the results (Fig. 5).

Detailed analysis was performed according to the intrastructural site of the elicitation and to the somatotopic manifestation of the responses, with special regard to the possible anatomic and functional relations. From the numerous data only two observations will be discussed, in view of their importance in respect of the extrapyramidal system.

The first observation concerns the tremor-influencing responses, i.e. the tremor-activating, inhibiting and rhythm or type-modifying manifestations. Depending on the parameters we observed the following.

The I parameter category seems to be the threshold stimulus mainly of the activating manifestation of the v.o.a., n.Sth. and Ra.prl., but simultaneously it is the threshold stimulus of the inhibitory response of the v.o.p. and Z.i. and even of the modifying responses of the v.o.p. and Ru.

The II parameter category proved to be the threshold stimulus mainly of the activating manifestation of the v.o.p. and of the inhibiting manifestation of the v.o.a., n.Sth. and of the rhythm or type modifying response of the Z.i.

The III parameter category was the threshold stimulus mainly of the activating responses of the Z.i., of the inhibiting responses of the Ra.prl. and even of the modifying manifestations of the structures not having responded hitherto in such a way (v.o.a., n.Sth., Ra.prl.).

To illustrate the above, let us review the observation in detail.

	Activating	Modifying	Inhibiting
I parameter category	v.o.a. (threshold) n.Sth. (threshold) Ra.prl. (threshold)	Ru. (threshold) v.o.p. (threshold)	v.o.p. (threshold) Z.i. (threshold)
II parameter category	v.o.p. (threshold) Ra.prl.	v.o.p. Z.i. (threshold)	v.o.a. (threshold) n.Sth. (threshold)
III parameter category	Z.i. (threshold) n.Sth.	Ru. v.o.a. (threshold) Z.i. v.o.p. n.Sth. (threshold) Ra.prl. (threshold)	v.o.a. v.o.p. Ra.prl. (threshold)
IV parameter category	Ra.prl.	v.o.a. v.o.p. Z.i.	v.o.a. v.o.p. Z.i.
V parameter category	n.Sth. Ra.prl.	v.o.a. v.o.p. Z.i. n.Sth.	v.o.a. Z.i.
VI parameter category		v.o.a.	v.o.a. v.o.p. n.Sth. Ra.prl. Z.i.

To facilitate survey, the structures and the pathways have been arranged beside each other as follows.

Category	v.o.a.	v.o.p.	n.Sth.	Z.i.	Ra.prl.
I	activating	inhibiting	activating	inhibiting	activating
II	inhibiting	activating	inhibiting	inhibiting	activating
III	inhibiting	inhibiting	activating	activating	inhibiting
IV	inhibiting	inhibiting	equalized	inhibiting	activating
V	inhibiting	equalized	activating	inhibiting	activating
VI	inhibiting	inhibiting	inhibiting	inhibiting	inhibiting

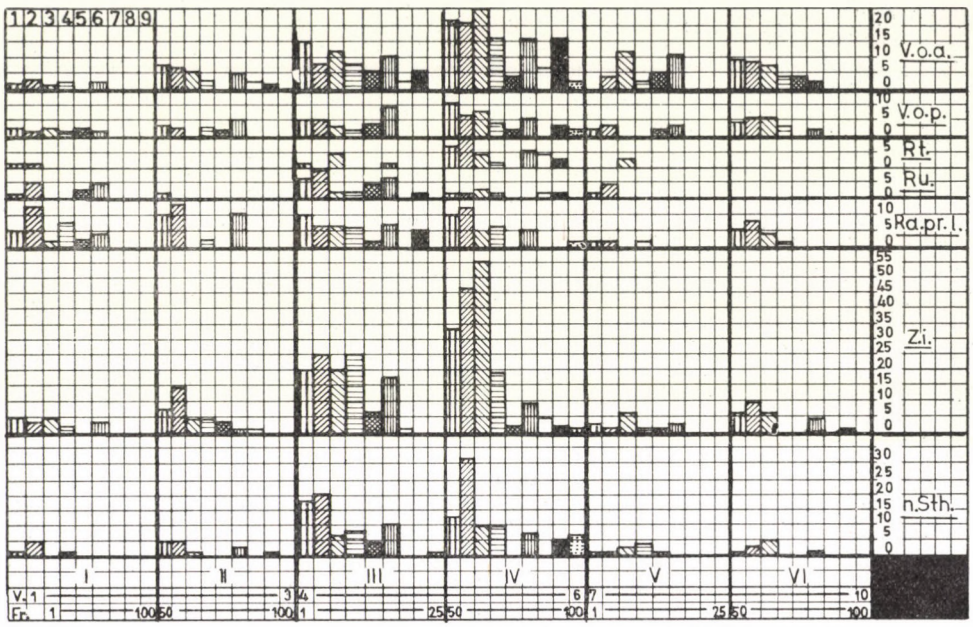

Fig. 5. Responses and their combinations in different structures (noted on right) according to the parameters (bottom I—VI; the average parameter categories, V; voltage of ES, Fr; frequency of the ES). The number at the right means the number of the responses. The number in the first columns means the type of the responses in every column i. e. 1—ocular movement, 2—somatomotor response, 3—inhibition, 4—activation of the other hyperkinesis, 5—alteration of the tremor rhythm or type, 6—sensation, 7—disturbance of speech, 8—vegetative response, 9—psychical response

The general inhibition in category VI represents the result of a stimulus which induces inhibition in every structure, or, else, of a stimulus which paralyzes activation in every structure.

At a low excitation level, v.o.a. and v.o.p. showed alternatively inverse while at higher levels, equally inhibiting manifestations. The n.Sth. showed identical or inverse manifestations with the v.o.a. and v.o.p. and even inverse manifestations with both, as if by its adaptation it would ensure a certain balance between the examined structures of the system, regulating thereby their cooperation. In the response manifestation of Ru., the responses modifying the rhythm and the type appeared to dominate.

In the subthalamic pathway system i.e. in Z.i. and Ra.prl., the predominance of opposite manifestations was observable in every parameter category, as if to ensure the transmission of a momentary interaction (inhibition, activation, rhythm and manifestation regulation) between the v.o. part of the VL nucleus and the n.Sth., or other structures. According to the above observations, the task of the Z.i. would be the transmission of inhibition and of some rhythm regulating impulses of the Ru., and the task of the Ra.prl.

rather the transmission of the activation and of the cooperation regulating impulses of the n.Sth.

Thus, we seem to have recognized interactions supposed to exist in the reverberating extrapyramidal circles, corroborating the fact that the structure manifestations are related to certain threshold and excitement levels and so the manifestations of different structures may be opposite or identical according to the requirements of the balance of interactions within the system. In Parkinson's syndrome a definitive inhibitory dominance was manifest which may characterize the pathologic change of interactions or may mean a new balance characteristic of Parkinson's syndrome (in other hyperkinetic conditions the dominant response was an activating one).

From the detailed analysis of the response it should be emphasized that lateral gaze, adversion, and mimic movements such as some grimaces, facio-vocal manifestations such as whistling, weeping and some hand movements — seeming to be physiological automatized movement patterns — were elicited mainly in the territory of the v.o.a., n.Sth. and Ra.prl. (where in other hyperkinetic conditions pathologic movement patterns were evoked). On the basis of this observation and the assumed relation between the structure's function and response manifestation, it may be concluded that the v.o.a., n.Sth. and Ra.prl. play a role in the elaboration and automatization of certain physiologic and pathologic movement patterns.

The majority of tremor-influencing responses were elicited from the v.o., Ru. and Z.i. The responses concerning speech and breathing, autonomous and psychic manifestations and the above mentioned observations suggest that the v.o.a. is the most manysided member of the examined structures.

The fact that the disturbances in extrapyramidal movements manifested themselves only in the wake state points to the role of the diffuse activating system. The stereotactic intervention must therefore be directed to that part of the activating system, which affects exclusively the motor disturbances, to the structures participating in their manifestation. According to our knowledge, such a selective intervention may affect the activating system at that point where the target lesion was effective and where a perturbation of the pathologic interaction of the structures responsible for the elaboration and automatization of the disturbance of movement are highly probable.

The practical conclusion of the response analysis, as regards the functional control of the electrode location in the subthalamus may be as follows. The location is adequate if the introduction of the electrode permanently stops the tremor, if low voltage low frequency stimulation induces or increases the tremor or modifies its rhythm or type, whereas high voltage high frequency stimulation stops it, and medium voltage and frequency do not elicit either a sensation (VPL) or movements. In the case of proportional or proportionally dominant tremors, our observations of the effect-somatotopy

provide a good orientation for completion of the lesion. As a final conclusion, the observations have allowed to assume that the extrapyramidal system is not a sensory system, nor a motor system as is generally supposed, but a system of organization and elaboration of the automatized psycho- and somato-motor patterns, supported by the informations of the sensory systems and manifesting itself in the motor system, ensuring and organizing the conditions for voluntary manifestations.

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HUMORAL MECHANISM OF ULCER-RESISTANCE OF THE ORGANISM ADAPTED TO PHYSICAL EXERCISE

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In earlier studies, blood sera obtained from sportsmen engaged in regular exercise were found to inhibit the production of peptic ulcer in rats. In the present study, the following effects of blood sera of exercise-adapted human subjects were demonstrable in the rat.

1) Inhibition of histamine-stimulated secretion of gastric acid;
2) inhibition of the response to histamine and serotonin in acceptor rats;
3) inhibition of the hypothalamo-pituitary-adrenocortical reaction to restraint.

It is suggested that the above phenomena as partial factors may be involved in the antiulcerogenic effect of serum.

It was shown earlier [5] that in the rat, regular swimming conferred a protective effect against various ulcerogenic factors. Statistical studies revealed a lower incidence of peptic ulcer in sportsmen under regular training than in persons of the same age groups abstaining from physical activity [6].

Ulcer-resistance of this kind was found to be transmissible. Serum obtained from humans and animals adapted to physical exercise showed an inhibitory effect on restraint and serotonin ulcer in rats and on histamine induced ulcer in guinea-pigs [7].

Likewise, responsiveness to histamine and serotonin was reduced in rats regularly made to swim, the animals exhibiting a slighter gastric secretory response to histamine and to pentagastrin than did the controls [2, 3, 4].

The next objective was to examine the antiulcerogenic properties of sera derived from organisms adapted to physical exercise. We had two possibilities for the study of this problem, i.e. to search for the active factor in the serum or to clarify the mechanism of its antiulcerogenic effect, identifying the factor(s) responsible for the effect in the acceptor organism. The present study has been carried out from the latter angle of approach.

The present experiments were based on the assumption that the ulcer-resistance of the acceptor organism was due to the same factors as have been demonstrated in the case of regular physical exercise. It was therefore by the study of gastric acid secretion and of the responsiveness to histamine and serotonin that we expected to throw light on the humorally transmissible ulcer-resistance. We also examined the effect of the sera in question on the hypothalamo-pituitary-adrenocortical reaction involved in restraint ulcer.

Material and method

The test group included 58 sportsmen in regular daily training, and a control group of 61 persons of similar age, all with sedentary occupation, abstaining from any kind of physical exercise. The laboratory animals to which the sera derived from these persons were administered, were female albino rats of identical breed, totalling 190 in number. The dose of the serum was invariably 5 ml by the intraperitoneal route.

Gastric HCl-secretion was studied by the method of HERR and PÓRSZÁSZ [10], the response to histamine and to serotonin, by the rectal temperature test.

For the induction of restraint-ulcer the animal under superficial anaesthesia was fixed to a rat bench with adhesive tape. Plasma steroids were estimated by the method of GUILLEMIN [9]. Results were evaluated by Students's *t*-test.

With the exception of the restraint-ulcer series, all tests were performed 6 hours after administration of the sera.

Results

In the first series of experiments the effect of the serum of trained and of inactive human subjects on the gastric secretory response to 1 mg/100 g histamine was studied. In the animals treated with the serum of trained subjects, gastric acid secretion was significantly less than in the untreated controls or in those treated with normal sera. The difference between the controls and those treated with normal serum did not reach statistical significance (Fig. 1).

In the subsequent series, we examined the influence of sera on the histamineinduced reduction of rectal temperature. In the rats injected with serum from exercise-adapted subjects, the hypothermic response to 2 mg/100 g histamine was significantly less weaker than in those treated with normal serum or in the untreated controls (Fig. 2).

The hypothermic effect of 0.6 mg/100 g of serotonin creatinine sulphate was influenced by the sera in a similar manner as that of histamine. Though the difference between exercise-adapted and inactive subjects was significant as early as at 45 minutes, at this time the serotonin had still a hypothermic

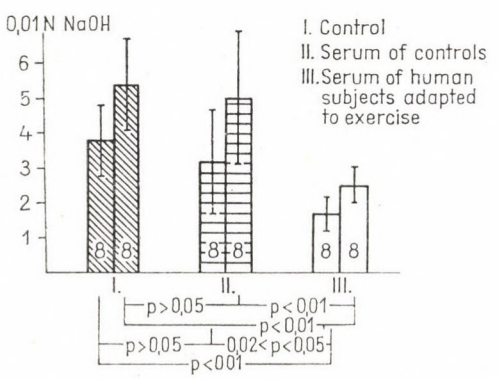

Fig. 1. Effect of serum of subjects adapted to exercise and of control subjects, on gastric acid secretion in rats

effect. At 90 minutes the hypothermic response ceased more rapidly than in the other groups (Fig. 3).

In the next series the hypothalamo-pituitary-adrenocortical response was studied. As the most suitable indicator of stress, the plasma steroid level was estimated. In the interest of comparability with the result of our earlier

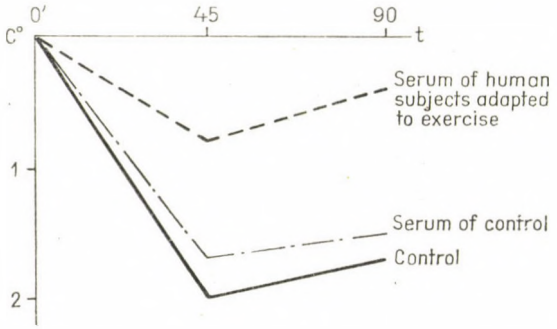

Fig. 2. Effect of serum of subjects adapted to exercise and of control subjects on the hypothermic response to histamine. Abscissa: time in min. after histamine administration. Ordinate: fall in temperature in °C

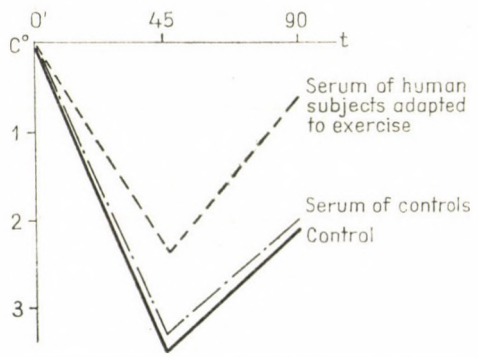

Fig. 3. Effect of serum of subjects adapted to exercise and of control subjects on the hypothermic response to serotonin

studies, the measurements were performed 16 hours after serum administration.

The blood sera were found to reduce the intensity of stress response. Though the animals which had been injected with sera of exercise-adapted donors exhibited a slight but statistically significant elevation, this was less marked than in the controls or in the animals injected with normal serum (Fig. 4). The ulcer-index showed a response similar to that observed in our previous studies.

The stomach of the rats treated with serum from exercise-adapted subjects either revealed no sign of abnormality or only ulcers of minor sever-

ity. Normal serum was also found to confer some degree of antiulcerogenic protection. In sum, the plasma corticoid level seemed to be related to the severity of the ulcer if the overall figures are considered. This, however, was not valid for the individual values which failed to reveal any close relationship with the severity of ulcer.

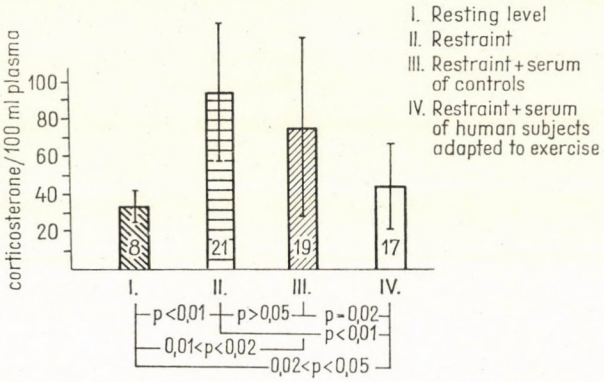

Fig. 4. Effect of serum of subjects adapted to exercise and of control subjects on the stress reaction in restraint-ulcer

Discussion

The present study has been based on the assumption that the anti-ulcerogenic effect of the humorally transmissible serum factor involves the same mechanism as in the donor organism. The results have shown this to be true. The serum of exercise-adapted subjects was found to reduce gastric acid secretion and to counteract the hypothermic effect of histamine and of serotonin. Our failure to demonstrate any change in the ATP contents and activity in the gastric mucosa in response to the administration of the sera under study may be regarded as negative evidence in support of our claim. Earlier studies also revealed a similar ATP concentration in the gastric mucosa of the controls and of the animals subjected to swimming exercise [2].

As regards the question whether the serum factor exerts its anti-ulcerogenic effect by a direct mechanism or whether it acts indirectly by producing certain changes through which the antiulcerogenic antihistamine and anti-serotonin mechanisms take effect, it is the second alternative which the present results seem to support, particularly in view of the fact that the tests have been performed 6 hours, and in the group of restraint-ulcer even as late as 16 hours, after the administration of the sera. However, the results do not permit to rule out the possibility of a direct effect or of an involvement of other potential serum factors connected with adaptation to exercise. We have actually found significantly lower serotonin levels in the serum of animals

adapted to physical activity than in those of the controls. Serotonin-free blood-sera derived from organisms adapted to muscular exercise displayed an inhibitory effect on serotonin-induced gastric contractions *in vitro*, in contrast to normal sera which showed no activity of this kind.

The serum of trained subjects was found to counteract the stress-response to restraint. On the other hand, no close relationship was demonstrable between the severity of ulcers and of the intensity of the stress in the individual animals. ADER [1] observed no close parallelism between the diurnal rhythm of the plasma corticoid level and of ulcer severity in rats if the restraint was carried out in accordance with the physiological variation of the corticosterone level. GRAY [8] regards the steroids as conditioning or permissive factors in ulcerogenesis. The significantly increased endogenous steroid levels demonstrable in the control animals may well represent one of the possible factors of restraint ulcer. It is, however, likewise possible that the high steroid level is merely an indicator of the hypothalamo-pituitary-adrenocortical response and that the mechanism of the ulcerogenic effect of the stress-reaction takes place through entirely different pathways. Sera obtained from exercise-adapted organisms were found to suppress the endocrine reaction, and in those animals where the steroid level was significantly elevated, the antiulcerogenic effect might have been due to other mechanisms referred to above. On closer analysis of the plasma steroid level in the control animals, together with those of the rats treated with the serum of trained donors, it would thus seem that the altered stress reaction, manifesting itself with a slighter increase in the plasma steroid level, fails to account in itself for the antiulcerous mechanism. On the other hand, it may well be involved, in association with other humoral factors, in the antiulcerogenic effect of muscular activity.

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FORMATION AND DEVELOPMENT OF MYELOID ELEMENTS IN THE FOETAL RAT FEMUR

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The ultrastructure of developing granuloid elements in the femur was studied in 16- to 21-day-old rat foetuses, the tissues being fixed in glutaraldehyde + osmium, contrasted with uranyl acetate + lead citrate and embedded in Durcupan ACM.

The femur exhibited intensive ossification on the 16th day of intrauterine life. Intercapillary and intracapillary medullary islets between the diaphyseal trabeculae appeared between the 17th and 18th day. Myeloid differentiation proceeded rapidly, numerous cells attained the promyelocytic, myelocytic and even metamyelocytic stage of maturation by the 18th day. The structure of these precursor cells differs, however, from that of the adult types, undifferentiated nuclei (marginal heterochromatin, well-developed nucleolus) being found together with a less intensive granulogenesis (paucity of azurophile granules; scarce, distended endoplasmic reticulum and Golgi cisternae). From the 20th day onward numerous fully differentiated granulocytes displaying a perinuclear halo, dense-core vacuoles as well as secondary and tertiary granulation, were observed.

Parallel with the neutrophile cells, eosinophile and basophile granulocytes appeared early in the femur, and the embryonal divergence of all these granules could be followed to the early blast stage.

In a previous study of the ultrastructure of medullary cells of 21-day-old rat foetuses and of adults rats (BUKULYA and BALÁZS, 1968) the foetal bone marrow revealed a fair number of blast cells and basophile granulocytes with sparse eosinophiles. The transitional precursors in the neutrophile series (promyelocytes, myelocytes, metamyelocytes) were found to differ in ultrastructure from the adult cell types.

The aim of the present work was to study at which stage of foetal life the granuloid cells are formed and to establish the ultrastructural features marking their early morphogenesis.

Material and methods

The bone marrow of randomly bred CFE-rats was studied between the 14th and 21st day of foetal life, two foetuses of male, two of female sex, each from different mothers, being worked up daily. The femur and tibia were prepared free from the muscles, both epiphyses were cut off and a longitudinal incision was made into the bone with a razor blade. The specimens were fixed for light microscopy in Helly's fixative, and for electron microscopy in 4.5% glutaraldehyde dissolved in 0.1 M Na-cacodylate buffer pH 7.2 at 4 °C for 1 hr. For foetuses younger than 17 days, a 0.05 M buffer was used. Beyond the 18th day, the bone marrow was detached from the bone with a razor blade under a Cytostat stereomicroscope and fixed for another 1 hr. The tissues were washed overnight with Na-cacodylate buffer of identical molarity

at 4 °C, post-fixed with a similarly buffered 1% OsO_4 solution for 1.5 hrs, washed again for 10 minutes, dehydrated in an ethanol series and embedded in Durcupan ACM. Sections 200 Å thick were cut with a Reichert Om U2 ultramicrotome placed on a formwar-coated, carbon-stabilized copper grid, contrasted in uranyl acetate + lead citrate and examined with a JEM 6AS electron microscope.

For light microscopy, the specimens were fixed for 24 hrs, washed in tap water, embedded in paraffin, cut at 5 μ and stained with haematoxylin-eosin or with Giemsa's dye.

The maximum deviation from the foetal ages specified above was 12 hrs.

Results

On the 16th day of intrauterine life, rat foetuses exhibit signs of intensive femoral ossification.

The first myeloid elements are demonstrable on the 17th to 18th day in the intracapillary and intercapillary areas of the newly formed intertrabecular medullary islets. They are blast cells of an ultrastructure prevalently similar to that of adult cells (Fig. 1), their nuclei being characterized by a marginal distribution of heterochromatin and by a well-developed nucleolus. The cytoplasm contains polysomes, mitochrondria with prominent cristae (Fig. 2) and endoplasmic reticulum in abundance. The Golgi system is usually well developed and transverse sections of the centrioles are usually detectable in their proximity (Fig. 3).

Mature granulocytes identical in structure with adult cells are still absent on the 18th day. The few transitional precursors demonstrable at that time, many of them myelocytes, are often of intracapillary localization. On the 20th day there is already a fair number of differentiated granulocytes identical in structure with those of adult animals. The lobulated nucleus contains plenty of heterochromatin and is surrounded by a halo (Fig. 4). The cytoplasm is rich in fine heteroform (secondary and tertiary) granules, there are plenty of dense-core vacuoles and cells of this kind may occasionally show numerous transverse sections of endoplasmic reticulum.

As regards the order of foetal morphogenesis, the incipient as well as the end forms of granulopoiesis are identical with the adult types (blast cells) on the 17th to 18th day and differentiated granulocytes between the 19th and 20th days. Follow-up of the order of differentiation of the transitional precursors was, however, made difficult by the following circumstances.

(a) Between the 18th and 19th day, a rapid and asynchronous differentiation is in progress, recognizable by the simultaneous presence of cells belonging to different compartments.

(b) The embryonal granuloid precursors are marked by a structural heterochronism. Organelles occurring in adult cells of different compartments may be found in one and the same foetal cell, as seen in Figs 6 to 8. In contrast to the cells of adult animals characterized by an abundance of granules attaining the size of 300 to 600 mμ, the ultrastructural pattern of young precur-

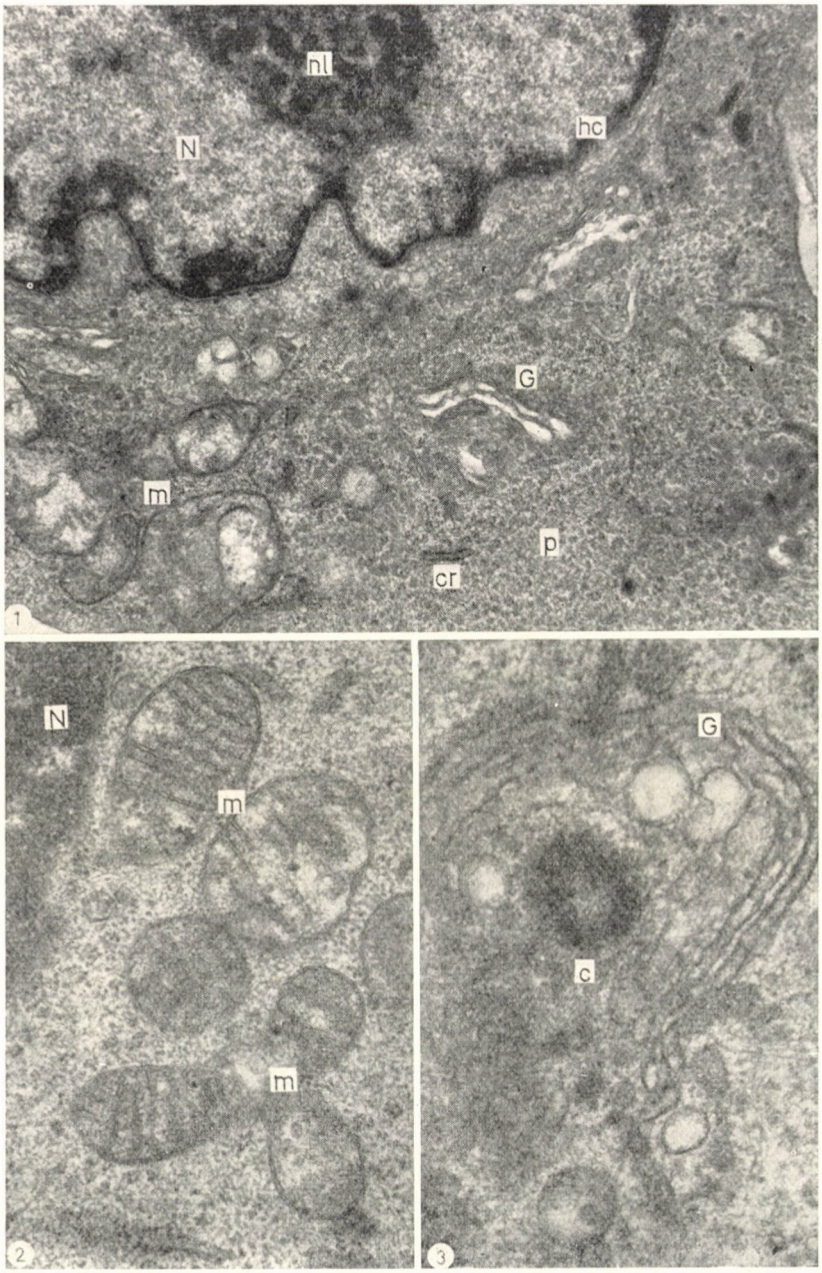

Fig. 1. Blast cell with marginal heterochromatin, differentiated nucleolus and numerous cytoplasmic polysomes. Bone marrow of an 18-day-old rat foetus. ×14,000. c = centriole, ch = chromosome, dv = dense-core vacuole, db = dense body, er = endoplasmic reticulum, f = fibrils, g = granule, G = Golgi apparatus, h = halo (perinuclear halo), hc = heterochromatin, l = lipid droplet, m = mitochondrium, mb = multivesicular body, mf = microfilament, N = nucleus, nb = nuclear body, nl = nucleolus, o = osmiophile body, p = polysome, ph = phagosome, pv = pinocytotic vacuole, v = vacuole.

Fig. 2. Mitochondrium-rich cytoplasm. Detail of a blast cell. Bone marrow of a 19-day-old rat foetus. ×34,000

Fig. 3. Differentiated Golgi region and centriole in a medullary blast cell of a 19-day-old rat foetus. ×54,000

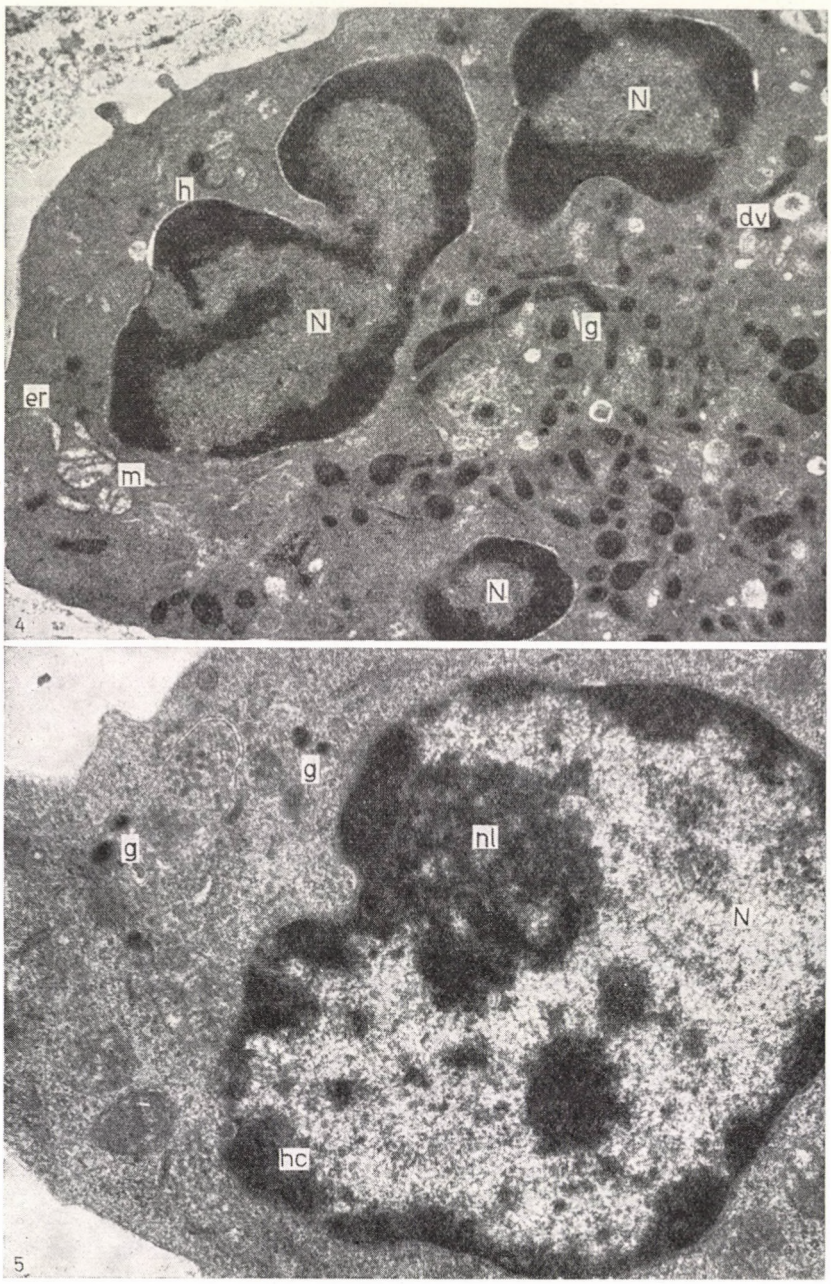

Fig. 4. Mature granulocyte from the bone marrow of a 19-day-old rat foetus. Lobulated nucleus with perinuclear halo, dark cytoplasm with abundant secondary and tertiary granulation. ×22,600

Fig. 5. Transitional granuloid precursor in the bone marrow of a 20-day-old rat foetus. The young nucleus contrasts with the fine cytoplasmic granulation characteristic of more differentiated cells. ×16,600

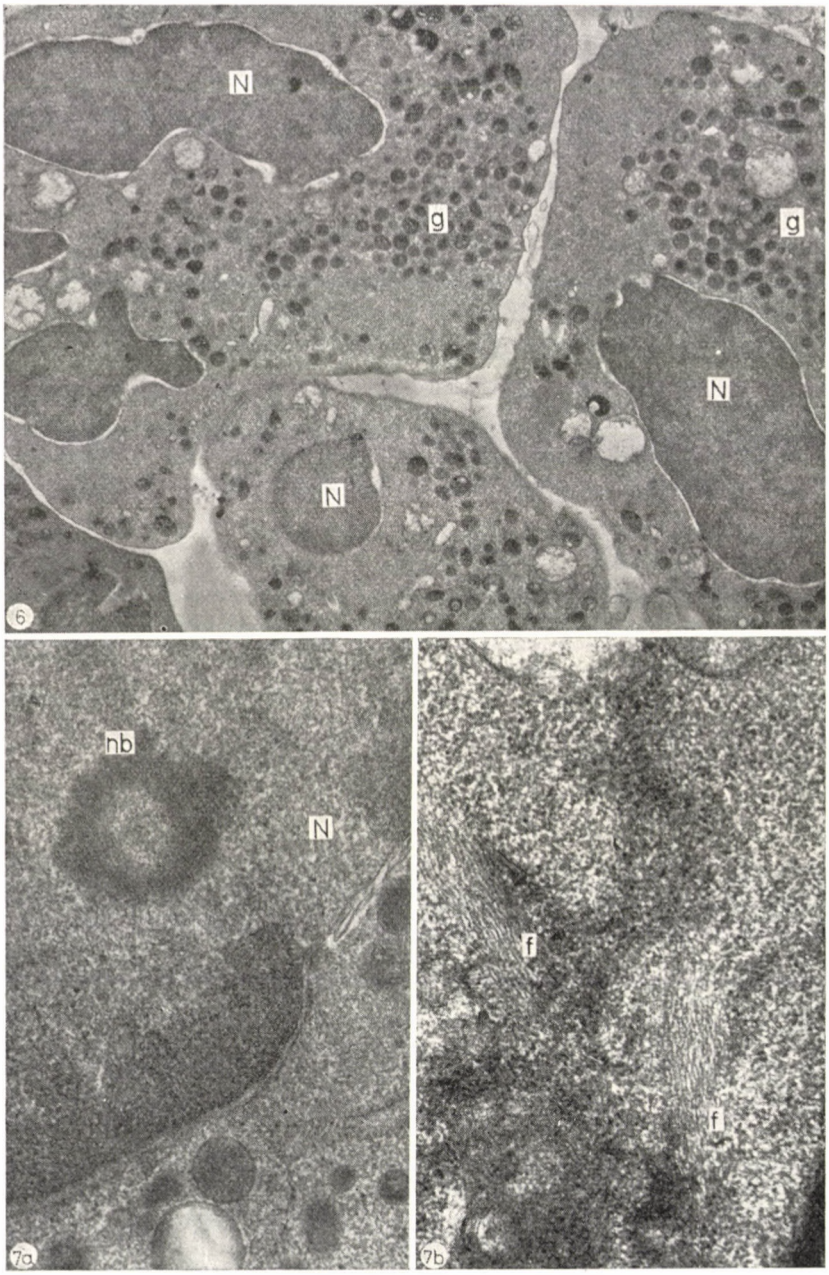

Fig. 6. Primary granules together with a lobulated nucleus and a perinuclear halo characteristic of differentiated cells. Transitional precursor from the bone marrow of a 21-day-old rat foetus. ×11,900

Fig. 7a. Nuclear body in a promyelocyte of an 18-day-old rat foetus. ×40,000

Fig. 7b. Fibrils in a medullary myeloblast of a 21-day-old rat foetus. ×40,000

sors (Fig. 5) is by no means incompatible with the presence of small and sparse granules. On the other hand, some cells with immature nuclei may exhibit a granulation characteristic of more differentiated forms. Again in other cases (Fig. 6) the cytoplasm of cells with nuclei of fairly advanced differentiation may show younger large granules.

(c) Further difficulties in ascertaining the order of differentiation are posed by the occurrence of organelles in foetal granuloid cells which may occasionally occur in cells of adult animals, as for instance the nuclear body in the promyelocyte of an 18-day-old foetus shown in Fig. 7a, or by the presence of fibrils in the cytoplasm of blast cells (Fig. 7b).

Formation of granules is most conspicuous alongside the Golgi-cysternae (Fig. 8a). The first granules are either clear particles of homogeneous structure or minute dense bodies not larger than 200 mμ $\varnothing$. In such cells transverse sections of rough endoplasmic reticulum are often seen (Fig. 8b). Granulogenesis may also involve the periphery instead of being confined to the Golgi region.

In the cell population of the 18th day of foetal life representing diverse stages of differentiation, all granule types characterizing the medullary cells of the adult animal are identifiable.

The eosinophils show signs of segregation as early as the 18th day. At this time young forms containing numerous basophile bodies are demonstrable (Fig. 9). On the 20th day a number of more differentiated eosinophils, i.e. metamyelocytes (Fig. 10) and granulocytes may be found in the bone marrow.

In accordance with the observations in adult animals, we have been able to trace back the genesis of eosinophile granules to the blast cell cytoplasm in foetal material. The incipient forms of eosinophile granules are small (not longer than 300 to 400 mμ) and of slight electron density (Fig. 11).

Young basophils were first detected on the 19th day of foetal life (Fig. 12a). Fig. 12b shows a differentiated basophil on the 21st day.

Mitoses are frequent in foetal material. Similarly to the interphase forms, young dividing granuloid cells are marked by a sparse granulation (Fig. 13).

Discussion

Embryonal differentiation of the erythroid, lymphoid and megakaryocytoid elements has been studied by numerous authors (Ackermann and Knouff, 1960; Bukulya, 1966; Grasso et al., 1962; Hagemann and Schmidt, 1960; Harland, 1940; Holyoke et al., 1969; Jones, 1960; Sanel, 1967; Zamboni, 1965). In contrast, there are few data concerning the embryonal morphogenesis of myeloid elements.

Plum (1943) traced the beginning of granuloid morphogenesis to the 18th, Jacobsen and Plum (1942) to the 20th day of foetal life. In an earlier

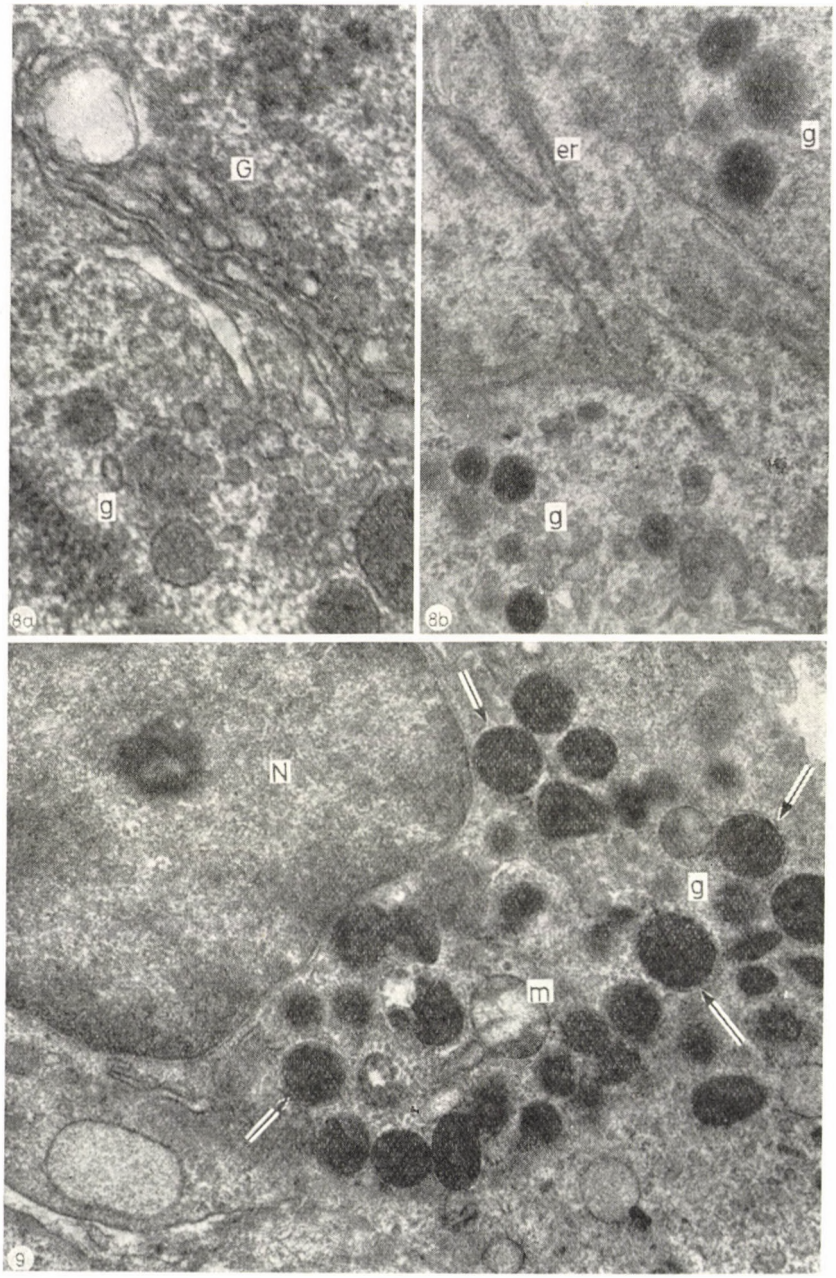

Fig. 8. Formation of primary granules in the myeloid cells of an 18-day-old rat foetus. The youngest granules, dense minute bodies with clear core, are seen in the Golgi region (a — ×59 400) or peripherally from it (b — ×40,400)

Fig. 9. Young eosinophil in the bone marrow of a 19-day-old rat foetus. The specific mature granules with a crystal core are found together with several basophile bodies (→) ×21,400

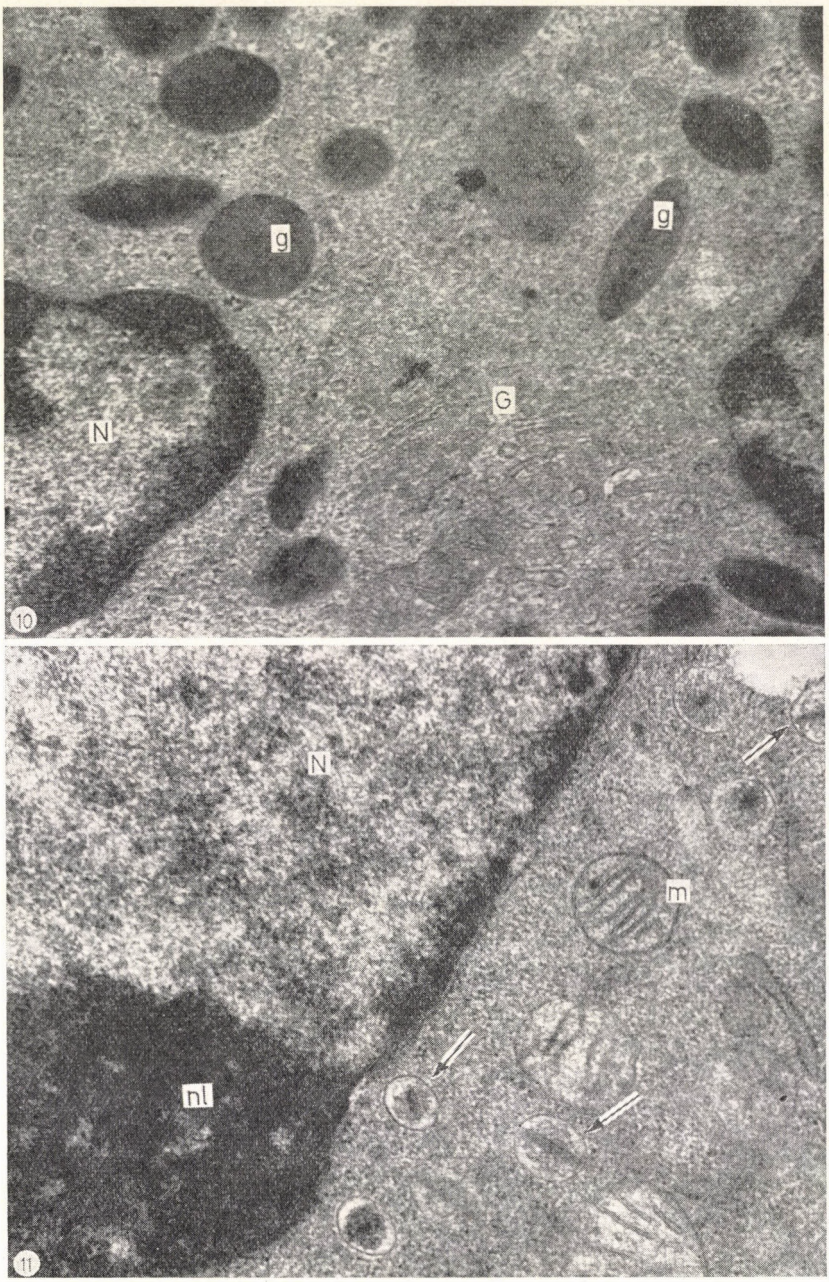

Fig. 10. Detail of a more differentiated eosinophil of a 20-day-old rat foetus. ×40,000

Fig. 11. Primary forms of eosinophil granules (→) in an embryonal blast cell. Bone marrow of a 21-day-old foetus. ×35,600

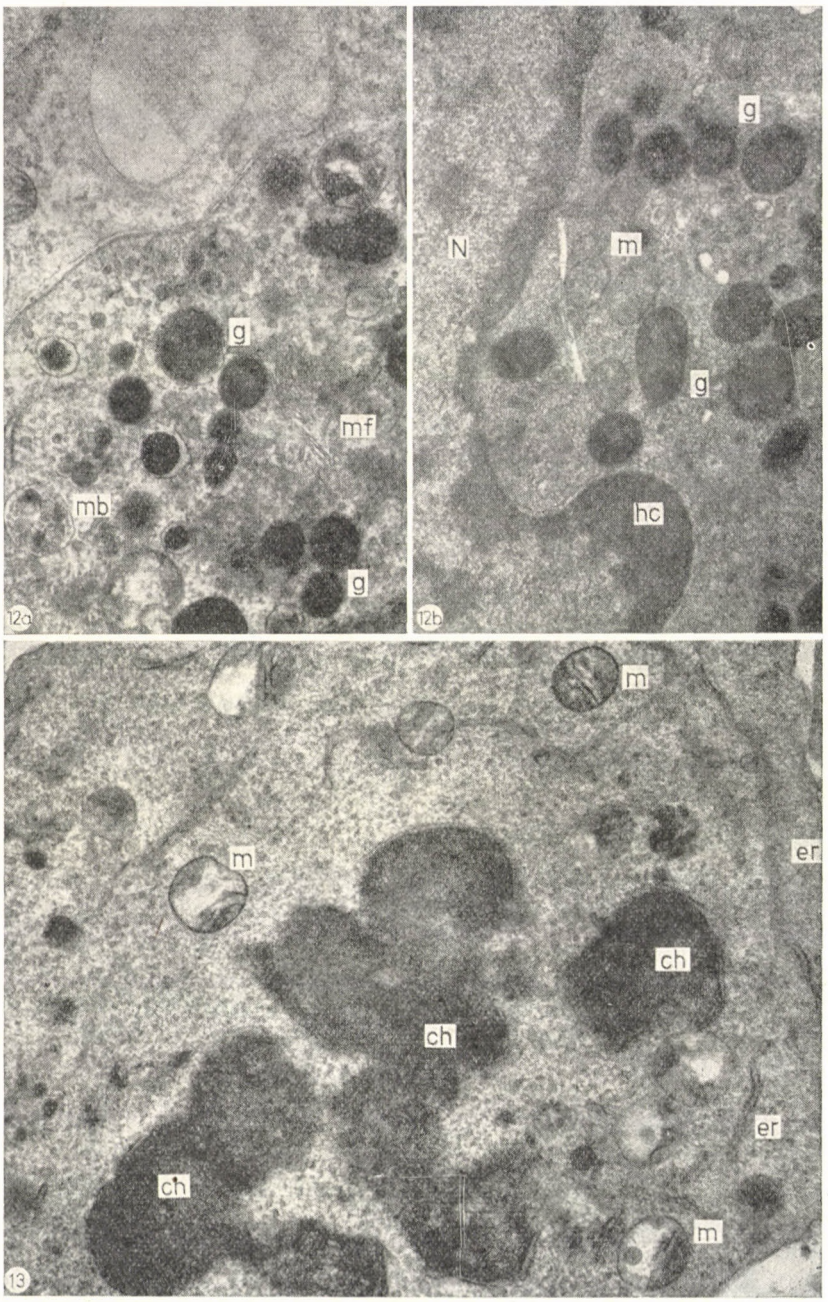

Fig. 12. a) Detail of a young basophil cell on the 19th day of foetal life. ×15,400. b) Detail of a mature basophil; 21-day-old foetus. ×22,700

Fig. 13. Dividing myeloid cell with chromosomes and sparse granulation, from the femur of a 19-day-old foetus. ×17,800

study we have been able to identify all types of differentiation from myeloblasts onward to mature granulocytes in the 21-day foetus by light and electron microscopy (BUKULYA and BALÁZS, 1968). In the present study it has been shown that myelogenesis in the femoral diaphysis begins on the 17th to 18th day of foetal life. In the epiphysis the process of differentiation starts later (KÖHLER, 1958); it is not until the 13th day of extrauterine life that the primitive bone marrow assumes the function of a blood forming organ.

Granuloid elements of advanced maturity may be found in the early stages of embryogenesis. It is true that these elements lack the criteria of differentiated granulocytes, but it must be borne in mind that embryonal haematopoietic cells may structurally differ from the cells of the adult organism (BUKULYA and BALÁZS, 1968), and younger forms, such as large nucleated erythrocytes containing little haemoglobin may also find access to the circulation (HAGEMANN and SCHMIDT, 1960). Therefore, the possibility that myeloid differentiation may start simultaneously or earlier in some other haemopoietic organ and that the repopulation of the bone marrow is secondary to this process, cannot be ruled out. Differentiation of the non-granuloid haemopoietic cells is believed to take place in the following manner.

The primitive blood cells, the haemocytoblasts, are the first to be segregated in the thymus, lymph nodes and liver, a process taking place in the rat on the 11th day of foetal life. Of these cells, the lymphoid elements differentiate first, i.e. in the rat on the 11th day (BUKULYA, 1966), in the mouse also on the 11th day (HOSHINO et al., 1969), in the rabbit's thymus and lymph nodes on the 17th to 18th day, in the rabbit's spleen on the 23rd day (HOSTETLER and ACKERMANN, 1969). Erythropoiesis starts somewhat later, in the rat's liver on the 13th day (HAGEMANN and SMITH, 1960), in the rat's spleen on the 17th day (HOLYOKE et al., 1966), in the rabbit between the 13th and 15th day (GRASSO et al., 1962), in man between the 70th and 100th day (ZAMBONI, 1965b; GRASSO et al., 1962). The megakaryocyte precursors are discernible in the rat liver between the 13th and 15th day, in the human liver between the 10th and 15th week (JONES, 1960; ZAMBONI, 1965a and b). ACKERMANN and KNOUFF (1960) identified primitive megakaryocytes on the basis of their strong PAS-positivity in the liver cells of 15 to 40 mm long pig foetuses before the appearance of megakaryocytoblasts.

Ever since the studies of MAXIMOW (1909) the possibility of an extramedullary genesis of myeloid elements has been brought up time and again in the literature. In the thymus of 10-week-old rats SYN and SAINTE-MARIE (1965a and b) demonstrated islets made up of neutrophile and eosinophile cells which on the basis of their distinctive features were found to cover the entire series of granuloid elements. As to embryonal cells, HARLAND (1940) noted early signs of granulocytopoiesis in the capsule and interlobular septa, and later in the cortex, of the rat thymus. In a paper of SANEL (1967) there

is an electron micrograph of the thymus of a 16-day-old mouse foetus with a portion of a myelocyte at the periphery. The observations of LATSINIK et al. (1970) relative to the pattern of haemopoietic cell series in liver tissue cultures derived from 16-day-old CBA foetuses are of particular interest. On explantation, 89% proved to be erythroid precursors, 7% of them haemocytoblasts, 2% myeloblasts, 1% promyelocytes and another 1% myelocytes. However, during culturing the proportion of erythroid precursors diminished in favour of the granuloid elements which increased their proportion from the initial 4% to 81% by the 5th, and to 98% by the 21st day. Changes in the colony-forming potency (CFU) of the embryonal cells were also noted. These facts and the results of our studies are well compatible with the possibility of an extramedullary embryonal granulopoiesis.

Our observations concerning primary granulogenesis mostly agree with data in the literature. According to ZAMBONI (1965b), the first granules demonstrable in haemocytoblasts and megakaryoblasts in the human foetal liver are actually minute, clear vesicles, occasionally with a dense core, evaginated and dissociated from the Golgi-system. The genesis of azurophile granules of myeloid cells of adult animals is similar; it occasionally takes place in cytoplasmic areas far outside the Golgi region (BESSIS and THIERY, 1961; BALÁZS, 1969).

Differentiation of the neutrophile, eosinophile and basophile granulocytes is complete at an early stage of development. On the 21st day of intrauterine life eosinophils are still sparse (BUKULYA and BALÁZS, 1968) despite the appearance of their precursors on the 18th day. On the 20th day, mature granulocytes of the same degree of differentiation as those of adults may also be found.

In our earlier studies of the myeloid elements of adult rats (BALÁZS and BUKULYA, 1968; BALÁZS, 1969) it has been shown that the myeloblast cytoplasm, light-microscopically of homogeneous appearance, actually contains incipient forms of eosinophile granules of slighter electron density and smaller volume than the granules of differentiated eosinophils. On the evidence of the present study this is valid also for embryonal cells.

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COAGULATION DEFECTS IN GLOMERULONEPHRITIS AND THE NEPHROTIC SYNDROME

By

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Blood coagulation was studied in 70 cases of acute, in 4 of subacute, in 34 of chronic glomerulonephritis and in 20 of nephrotic syndrome; 780 coagulation studies involving 16 different procedures were carried out. Close attention was given to the clinical course.

Hypercoagulability was invariably demonstrable at the onset of typical acute, and during acute exacerbations of chronic glomerulonephritis. The signs were more marked in the nephrotic syndrome whether of the primary type or secondary to amyloidosis, diabetic glomerulosclerosis or systemic lupus erythematosus. A case of Goodpasture's syndrome was likewise associated with hypercoagulability though an antithrombin factor of heparin character was demonstrated just before death. Hypocoagulability was most frequent in inactive chronic glomerulonephritis. On the other hand, hypercoagulability may be interpreted as a sign of activity in nephropathies, and its persistence is an adverse prognostic sign.

Anticoagulants failed to produce any decisive change in 8 patients with glomerulonephritis or the nephrotic syndrome.

Production of fibrin clots in the glomerular loops in human glomerulonephritis, occasionally even in large numbers (Reichel-type) has long been noted by pathologists, and it has also been demonstrated in the glomerular lesions of rats [19] and dogs [24] treated with nephrotoxic serum. We have presented indirect evidence of coagulation defects in nephrotoxin-treated rabbits. In the successive stages of the process, gelatin-stabilized India-ink was injected intravenously according to JANCSÓ. On treatment with very potent nephrotoxin, India-ink thrombi plugging the small vessels were demonstrable in the renal cortex. Precipitation of the exogenous colloid was interpreted as a sign of latent coagulopathy [11, 12, 13]. On the grounds of these findings, we have now studied the coagulation defects associated with glomerulonephritis and the nephrotic syndrome. Our first results were reported in 1967 [4]. The question has been investigated simultaneously by numerous other authors and various coagulation defects were described [1, 5—8, 10, 16, 18, 20—23, 25].

The present work was carried out to study the relationship between coagulation defects and the clinical course of renal disease.

Material and methods

In 128 patients, 780 coagulation studies were performed. Of the patients, 70 had acute, 4 subacute and 34 chronic glomerulonephritis and 20 were nephrotic. The following 16 laboratory tests were performed.

1) Bleeding time, according to DUKE;
2) Whole blood clotting time, according to LEE and WHITE;
3) Platelet count by phase contrast microscopy, according to BRECHER and CRONKITE;
4) Clot retraction, according to BIGGS and MCFARLANE;
5) Coagulation time of recalcified plasma, according to HOWELL;
6) QUICK's prothrombin time;
7) Prothrombin consumption;
8) Study of serum coagulation accelerating factor, according to HORN, KOVÁCS and ALTMANN;
9) Thrombin time;
10) Thrombin clotting time in the presence of toluidine blue (Toluidine blue time);
11) Thrombin inactivation time, according to GERENDÁS;
12) Fibrinogen gravimetry;
13) Euglobulin lysis time, according to VON KAULLA and SCHULTZ;
14) Partial thromboplastin time;
15) Thromboplastin generation test, according to BIGGS and DOUGLAS;
16) Thromboelastography according to HARTERT.

We primarily relied on the plasma fibrinogen level, euglobulin lysis time and on thromboelastography (TEG). The hatched areas seen in the diagrams correspond to the scatter of the normal values. The normal thromboelastogram is represented by a broken line. Maximum elasticity of thrombus (mε) has also been represented. Measurement of partial thromboplastin time and the thromboplastin generation test were confined to cases involving particular problems. The coagulation tests were supplemented with capillary tests (LANDIS, GÖTHLIN, RUMPEL-LEEDE, BORBÉLY).

The dynamics of the process responsible for the primary disease was checked on the grounds of several parameters, as seen in the diagrams. The most important of these included the Addis count [26], estimation of protein excretion by measurement of the amount of nitrogen, serum albumin, the serum complement titre [17], ASO-titre, GFR measured by routine endogenous creatinine clearance. In special cases, the clearance of inulin and PAH and the filtration fraction were also estimated.

Results

Fig. 1 summarizing the results shows that a normal coagulation was confined to a fraction of the patients, mostly after the cure of glomerulonephritis. The predominant coagulation defect in the various types of glomerulonephritis and nephrosis was a hypercoagulability, either isolated or together with signs of hypocoagulability. It is therefore the relationship between hypercoagulability and the clinical course which had first to be considered.

In the early stage of typical acute glomerulonephritis hypercoagulability was a regular finding. An illustrative case is given in the following.

The patient was a 19-year-old female, first seen on the 17th day of acute glomerulonephritis. The high fibrinogen level, the protracted euglobulin lysis time and the broad thromboelastogram were conclusive of hypercoagulability. Penicillin treatment resulted in an improvement of the condition, with the disappearance of gross haematuria, reduction in the Addis count of erythrocytes and in hypercoagulability. Even microscopic haematuria diminished to a minimum, and this could be considered a residual haematuria, the more so

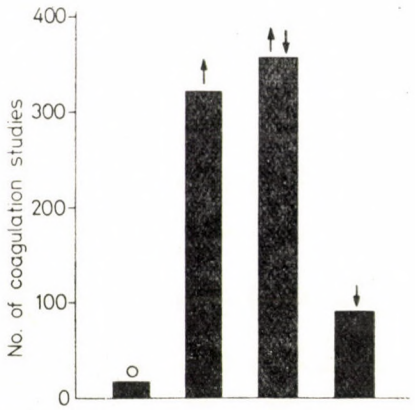

Fig. 1. **Results of coagulation studies in glomerulonephritis and in the nephrotic syndrome. ○ = normal coagulability, ↑ = hypercoagulability, ↓ = hypocoagulability**

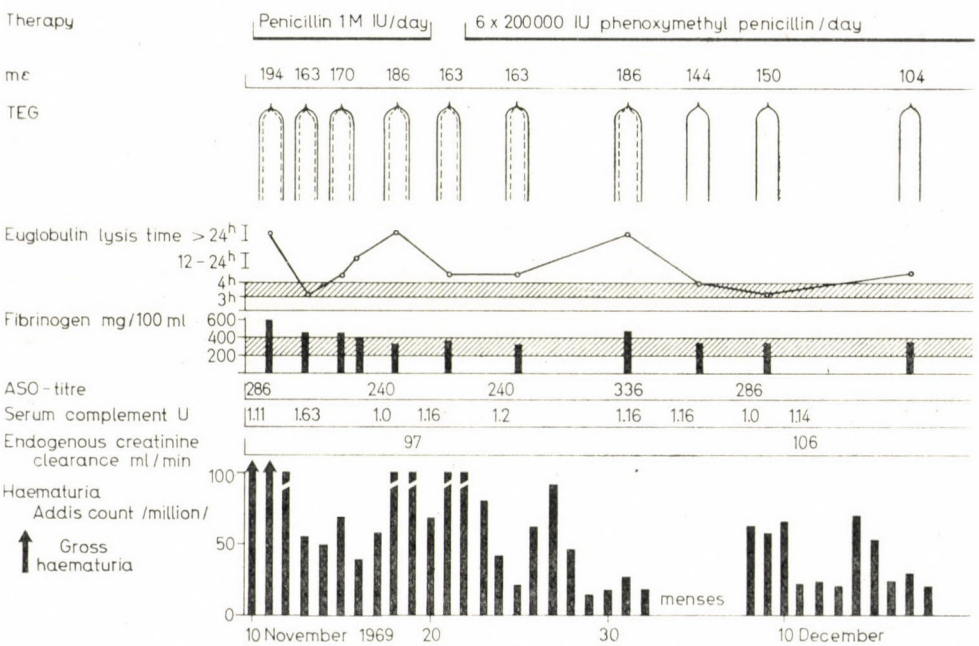

Fig. 2. **Coagulation defects at the onset of acute glomerulonephritis**

as endogenous creatinine clearance pointed to a normal glomerular function. At this stage, fibrinolytic activity was still reduced. This phenomenon which was observed in several similar cases may be regarded as a residual coagulation defect (Fig. 2). The opposite phenomenon, an enhanced fibrinolytic activity of plasma, may occur occasionally as a sign of a residual coagulation defect. In some very mild cases the fibrinolytic activity of plasma may be

increased early according to euglobulin lysis time, or any changes in coagulation may be absent.

Acute exacerbations of chronic glomerulonephritis are also accompanied by hypercoagulability.

The patient, a 27-year-old male had been under our care for years for chronic inactive glomerulonephritis. Apart from minor signs of hypocoagula-

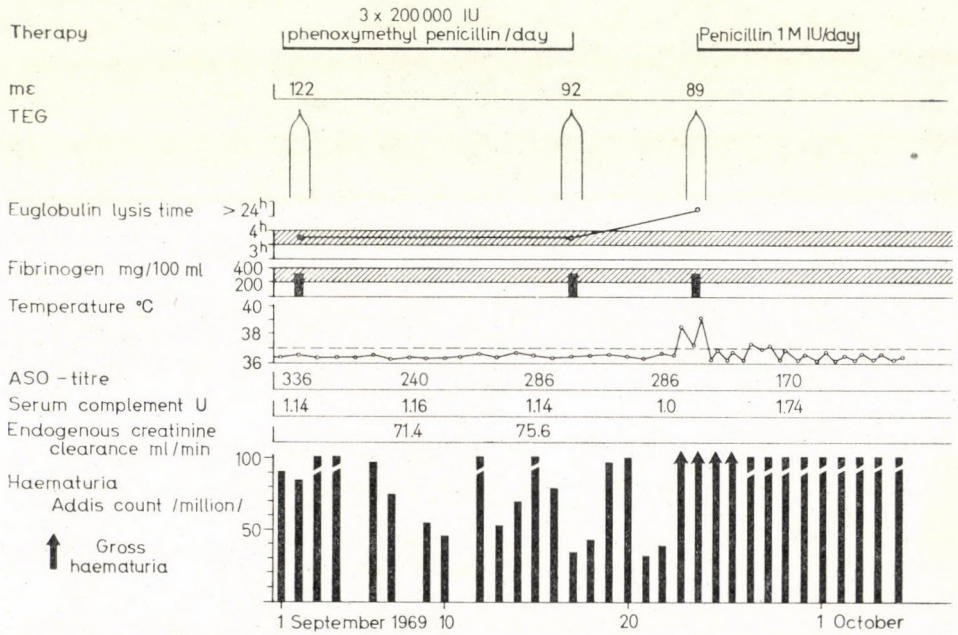

Fig. 3. Reduction in plasma fibrinolytic activity during acute exacerbation of chronic glomerulonephritis

bility no other clotting abnormality had been noted. He had been on a preventive scheme of oral penicillin which was discontinued on admission. A few days later he developed a sore throat with fever and gross haematuria. A pharyngeal swab yielded β-haemolytic streptococci. Here we were able to follow up the coagulation defect associated with an acute exacerbation from its earliest stage and thus to demonstrate a protracted euglobulin lysis time, i.e. a distinct reduction in fibrinolytic activity, within a few hours of the acute events. Thus, the earliest stage of blood coagulation disorders associated with the allergic process is an enhanced coagulability (Fig. 3).

The most conspicuous signs of hypercoagulability were found in the nephrotic syndrome, regardless whether the process was of the primary type or secondary to amyloidosis, diabetic glomerulosclerosis, systemic lupus erythe-

matosus, etc. On the evidence of our observations, hypercoagulability may be regarded as a sign of activity of the nephrotic syndrome.

One of the patients, a 28-year-old female, was admitted with a primary nephrotic syndrome. Administration of furosemide (Lasix, Hoechst, Frankfurt) induced a spectacular improvement, with the loss of 15 litres of oedema fluid in three weeks, as well as an increase in serum protein concen-

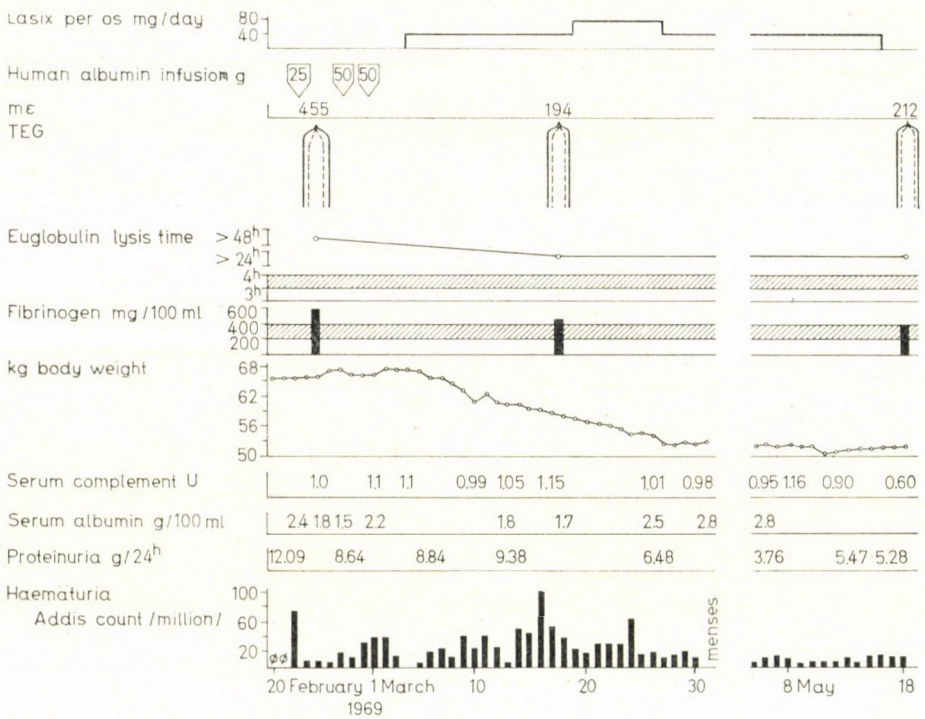

Fig. 4. Coagulation defects in primary nephrotic syndrome

tration and a fall of daily urinary protein excretion from 12 g to 3 or 5 g. However, blood clotting was still enhanced, as indicated by the thromboelastographic pattern and by the diminished spontaneous fibrinolysis. The serum complement titre was declining as a sign of immunological activity. Therapy had thus little influence on the coagulation defect, and in fact later an exacerbation ensued (Fig. 4).

Combination of symptomatic and immunosuppressive treatment may completely change the situation, as illustrated by the following case.

A 16-year-old male had been under observation for primary nephrotic syndrome, i.e. for "pure" nephrosis. An elevated plasma fibrinogen level, a reduced fibrinolytic activity together with a broad thromboelastogram had repeatedly been found prior to treatment. Administration of prednisolone

and subsequently of furosemide resulted in a loss of 20 litres of oedema fluid. Parallel with the clinical response hypercoagulability changed temporarily into hypocoagulability, as indicated by an abnormal narrowness of the thromboelastogram. The low serum complement level returned to normal and lasting remission ensued (Fig. 5).

The following case of amyloidosis represents the group where the nephrotic syndrome was elicited by some identifiable primary process.

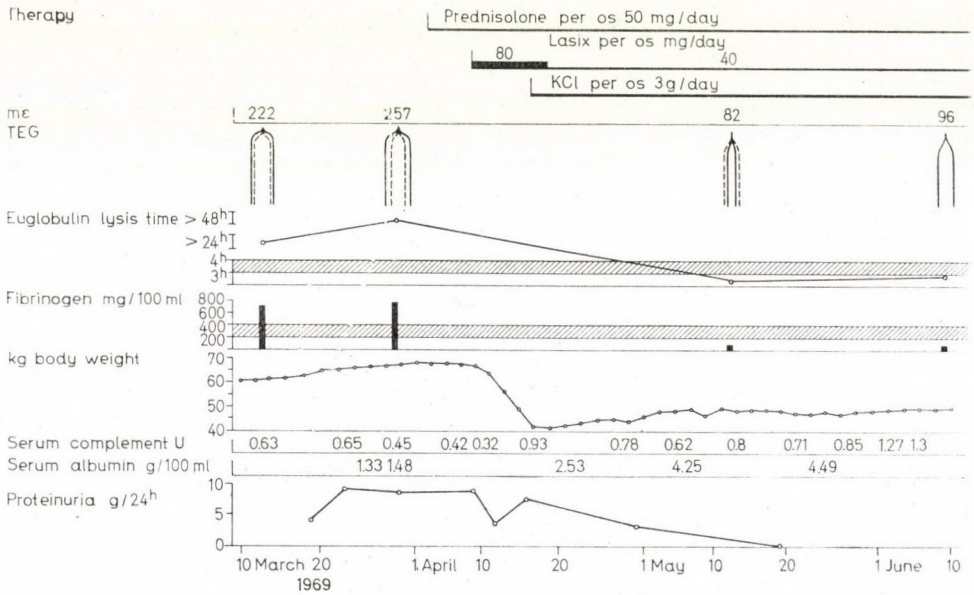

Fig. 5. Coagulation defects in primary nephrotic syndrome ("pure" nephrosis)

The patient was a 59-year-old female with clotting disturbances which had been investigated for the last months without showing any change. She was started on anticoagulant therapy. To acenocoumarol (Syncumar, EGYT, Budapest), the thromboelastogram showed a typical response. However, thus far we cannot yet assess the effect of anticoagulants in amyloid nephrosis, though persistent hypercoagulability belongs to the typical features of amyloidosis (Fig. 6).

One of the cases provided us with the opportunity of studying the coagulation disorders associated with Goodpasture's syndrome. The patient was a 19-year-old female. Fig. 7 sums up the results of investigations and the clinical features. The clinical course was marked by hypercoagulability as reflected by hyperfibrinogenaemia and an excessive reduction, if not an entire lack, of fibrinolytic activity and an abnormally broad thromboelastogram. The results have been represented diagrammatically according to GERENDÁS [9].

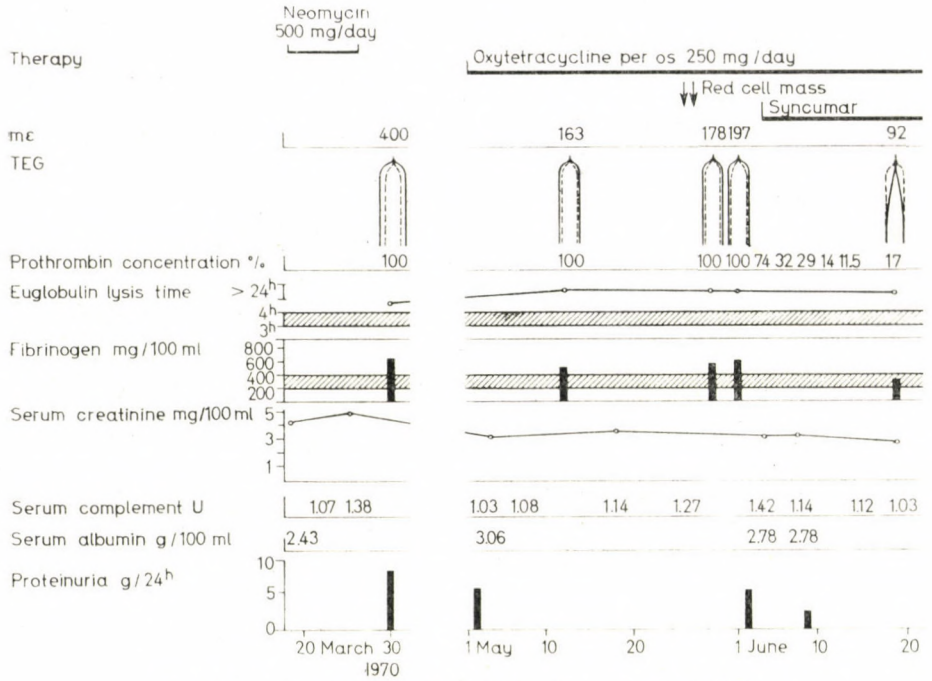

Fig. 6. **Persistent hypercoagulability in renal amyloidosis**

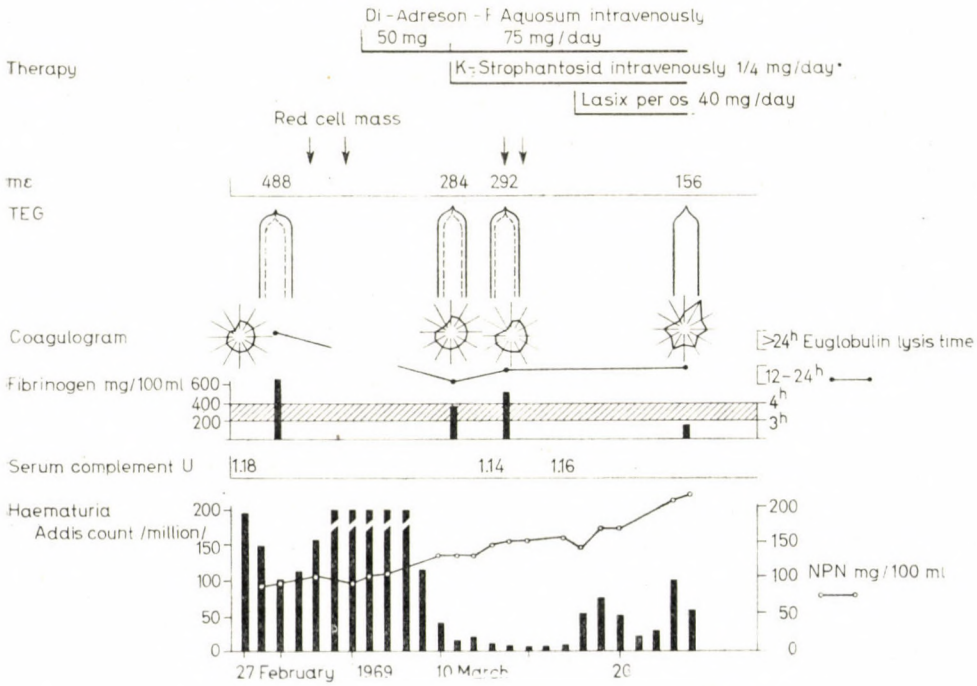

Fig. 7. **Coagulation defects in Goodpasture's syndrome**

On the evidence of the coagulogram performed before death the patient developed a bleeding tendency terminally, most probably under the influence of an antithrombin factor of heparin character. We have connected this finding with the terminal events. The suction cup test and the tourniquet test revealed an excessive capillary fragility. There is immunological evidence that Goodpasture's syndrome involves the formation of antibodies directed against the basement membrane, thus linking up the renal and pulmonary alterations with an autoimmune pathomechanism [2, 15]. On confronting these results with our findings it appears that the bleeding tendency in Goodpasture's syndrome is connected with an abnormal capillary permeability.

As to the hypocoagulability in this case, it is by no means an unfavourable sign as regards the activity of nephritis or nephrosis; it was usually observed in inactive chronic nephritis or after successful immunosuppressive treatment, as seen in Fig. 5.

Discussion

Hypercoagulability is a sign of activity of glomerulonephritis and nephrosis, particularly informative in the nephrotic syndrome. One of our patients had developed signs of hypercoagulability quite suddenly and went into a relapse soon afterward. In other words, the recurrence had been foreshadowed by an increase in blood coagulability, and this would call for the inclusion of coagulation tests into the follow up scheme in renal disease.

Persistent hypercoagulability is an unfavourable prognostic sign. We have lost 11 patients with glomerulonephritis or nephrosis in the last years. Hypercoagulability was confirmed by an increased plasma fibrinogen level, a reduced fibrinolysis, and a typical thromboelastogram in 9 patients and by two of these signs in 2 patients. On the other hand, hypocoagulability points to a favourable prognosis, as it did in fact in our cases of chronic glomerulonephritis of stationary nature.

These observations have their implications in the intriguing problem of the anticoagulant treatment of glomerulonephritis [11, 13]. Recently, BERLYNE and MALLICK [3] have suggested that ischaemic heart disease was one of the complications of the nephrotic syndrome, with an incidence 85 times as high as in the age-matched general population. We have also observed coronary occlusion in primary nephrotic syndrome. Two of our patients with Schoenlein—Henoch's purpura associated with glomerulonephritis developed digital gangrene; one of these patients died, the other had amputations of several digits.

These observations have prompted us to use anticoagulants in the diseases under discussion. However, in the 8 cases of glomerulonephritis or nephro-

sis where it had been employed, it failed to produce any improvement, though the clinical course of two patients certainly encourage further attempts. One of them, a male with systemic lupus erythematosus, developed vein thrombosis of right leg. He had been in a preuraemic condition when started on acenocoumarol. This treatment was continued for 18 months, until death. At necropsy, both renal veins, particularly the right one, were narrowed by organizing thrombi. The inference that without anticoagulant therapy occlusion of both renal veins would have inevitably ensued, almost suggested itself. In the other case, a patient with primary nephrotic syndrome, leg vein thrombosis recurred four times before anticoagulant therapy had been prescribed. Since two years he had no recurrence. Although the data in the literature are scarce [14] and our observations still too few to be conclusive, nevertheless, they point to the advantage of combined anticoagulant and corticosteroid or cytostatic treatment.

Finally, the question arises whether glomerulonephritis and the nephrotic syndrome are to be regarded as coagulopathies? This must be answered in the negative, since the coagulation defects fail to account for the entire syndrome, though they certainly do belong to it, in the same manner as oedema, hypertension or haematuria belong to the clinical pattern of glomerulonephritis.

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THERAPY OF AUTOIMMUNE NEPHROPATHY WITH STEROIDS AND CYTOSTATICS

A FOLLOW-UP STUDY OF 80 PATIENTS AND OF 16 YEARS

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The therapeutic aspects of lupus nephropathy — as the prototype of autoimmune nephropathies — are analysed in the light of sixteen years observations. The introduction of "immunosuppressive" therapy with 6-MP in 1963 marks a turning point in its hitherto generally fatal prognosis. With the use of haemodialysis and of kidney transplantation survival is possible even after total destruction of the kidney, however it is more reasonable first to apply the "immunosuppression" with appropriate cytostatics to avoid autoimmune nephropathy, than to prevent the rejection of a transplanted kidney.

Of the 250 patients with typical SLE observed between 1953—1968, 80 had lupus nephropathy. Of these the outcome of 11 is unknown, 36 died, 33 are still under observation.

This follow-up study of 16 years may be divided into 3 periods each of five years. During the first five-year period (1953—57) lupus nephropathy was the prevalent cause of death in SLE, which may be ascribed to the sporadic application of steroids and to the inadequacy of its doses. Antimalarial drugs applied in that period also failed to influence the renal process. Long-term intensive ("immunosuppressive") steroid therapy marking the second period (1958—62) was found to reduce the incidence of nephropathy in SLE and so to improve the prognosis. By the additional use of cytostatics from 1963 on, even the steroid-resistant cases have become curable. Since the demonstrability of the LE-factor or other diagnostic criteria of SLE affected neither the outcome, nor the clinical picture of autoimmune nephritis, the observations are entirely valid for the similar autoimmune nephropathies without the signs of SLE.

Involvement of the kidneys in systemic lupus erythematosus (SLE), the so-called lupus nephropathy, usually assumes the clinical features of genuine nephrosis or of primary chronic nephritis or of both. In its mildest form, the condition manifests itself with proteinuria without any other symptom. Nephropathy is regarded as the most therapy-resistant of all systemic manifestations of SLE, accounting, in fact, for the majority of deaths in this disease.

In the literature there are reports on a favourable response of lupus nephropathy to long-term massive steroid therapy [10, 12]. Our experience [6, 7, 8], in agreement with that of other authors [4, 11, 13, 14] have been much less favourable. It has been the induction of cytostatics into the treatment [6] which improved radically the rather gloomy prognosis of lupus nephritis.

As a result of the observation that in the course of the natural history of SLE, manifestations may be confined to a single organ for long periods [5],

certain issues of primary importance appear now in a new light. Identical isolated renal disease may thus well be due to the same immune pathogenesis, though obviously it is not referred to as lupus nephropathy but, as the case may be, usually as "chronic glomerulonephritis", or "nephrosis", or as an intermediary syndrome combining the two types, "Nephritis mit nephrotischem Einschlag" (nephritis with nephrotic features).

Twenty years observations have given ground to our impression that neither the outcome, nor the therapeutic possibilities in "lupus nephropathy" are affected by the variable positivity of the LE-cell phenomenon, i.e. the presence of the LE-factor in the antibody-spectrum and by the microscopic evidence of haematoxylin-bodies. If in a given case of nephrosis or chronic glomerulonephritis, the LE-factor happens to be demonstrable for some time, of course it facilitates to recognize the autoimmune nature of the basic process. By means of biopsy labelled anti-antibody techniques, electron microscopy, etc. should be used to identify the character of the autoimmune reaction, once its involvement has been established and to locate it within the structure of the kidney. Insight into these relationships has revealed that the therapeutic observations derived from the diverse typically autoimmune renal manifestations of SLE, are valid for other similar immunopathological types of nephropathies, even for those without any demonstrable signs of SLE.

In the present study, we have collected further evidence relating to the value of "immunosuppressive" (cytostatic) therapy in addition to our earlier report with low and high dose steroids [7, 14], in the light of 16 years observations of our clinical material of 250 subjects with SLE, 80 of them with lupus nephropathy.

Patients and treatment

A total of 80 patients with lupus nephropathy was observed since 1952/53. The 16 years elapsed since may be divided into three periods. In the first period, 1953—1957, therapy of SLE was confined to small-dose cortisone and to anti-malarial agents (mepacrine, chloroquine), their administration having been limited to dangerous exacerbations. In the second period, 1958—1962, a purposeful intensive "immunosuppressive" treatment was carried out by the use of prednisolone in every case, with the aim of attaining complete remission and of maintaining it by long-term therapy on the basis of a suitably organized follow-up scheme. In the third period from 1963 on, cytostatic agents (generally 6-mercaptopurine, and in a few cases azathioprine, cyclophosphamide, chlorambucil) were used, in addition to the high dose steroid medication referred to above.

Dosage of the cytostatic agents was adapted to the individual case. The general rule was to attain the maximum dose which, while ensuring the desired therapeutic effect would still not damage the haemopoietic organs during the treatment.

Results

Distribution of the patients over the three periods is shown in Table I. It can be seen that, since the use of steroids in "immunosuppressive" doses, regardless of the presence or absence of nephropathy at the onset, the incidence of lupus nephropathy has been declining, not only in relation to

the total series but also to the number of new cases, and its decline continued over the third period, marked by a consistent cytostatic therapy in steroid-resistant nephropathies. The data relative to the two earlier periods [7] serve here as a basis for comparison with the results obtained during the third period.

Table I

Treatment of lupus nephropathy between January, 1953, and December, 1968

Diagnosis	I 1953—1957 Corticosteroids, small-doses, sporadic use	II 1958—1962 Corticosteroids, massive doses, long-term use	III 1963—1968 Corticosteroids + cytostatics	Total number of cases
SLE	53 (21.2%)	117 (46.8%)	80 (32%)	250 (100%)
Nephropathy	28 (52.8%)	32 (27.2%)	20 (25%)	80 (32%)

Table II

Lupus nephropathy: 36 fatal cases and 33 survivors. Distribution of cases and duration of disease

Sex	Number of cases		Average age (years)		Average duration of disease (years)			
					Deaths		Survival	
	Death	Survival	Death	Survival	Between the first manifestation of SLE and death	Between the first renal manifestation and death	Since the first manifestation of SLE	Since the first renal manifestation
♀	26	26	36 (16—64)	37 (14—69)	4	3	8	6
♂	10	7	41 (28—67)	30 (17—55)				

We have found that 1) massive-dose "immunosuppressive" corticoid therapy reduced the incidence of nephropathy in the course of SLE, and 2) steroid-resistant nephrosis is responsive to suitable cytostatic drugs in the majority of cases, to the degree of full remission, and possibly even of a permanent cure.

Since 1953, 36 of the 80 patients with lupus nephropathy died, 33 remained under our continuous control and the fate of 11 is unknown. Most patients are young females. In the fatal cases, mean survival after the first manifestations of SLE was 4 years and after those of nephropathy, 3 years. In a number of cases nephropathy was demonstrable at the time of the first manifestations of SLE. For the survivors, the respective times correspond to 8 and 6 years (Table II); up to 1968, survival in this group was twice as long as in the fatal cases.

These facts clearly show that intensive steroid therapy is apt to bring the process to a halt, and supplementary cytostatic therapy, to induce full remission (Table II).

It was chiefly in the nephrosis syndrome where cytostatic therapy elicited a prompt response. Even in cases of major severity, marked by extensive oedema, polyuria, often by isosthenuria and by non-protein nitrogen levels over 60 mg per 100 ml, the effect of adequate doses was manifest in three weeks. Recovery of concentration capacity and relief of oedema ensued; proteinuria responded less readily.

Table III

Treatment and mean survival in fatal cases of lupus-nephropathy

	Treatment	Number of cases	Mean survival (years)
1	Sporadic administration of corticosteroids in small doses (40 mg daily) + antimalarial drugs	19	2.5
2	Long-term administration of corticosteroids in large doses (50 mg daily)	10	6.0
3	Corticosteroids (40 mg daily) + 6-MP (50 to 100 mg daily)	5 17	3.5 5.0
4	Corticosteroids (60 mg daily) + 6-MP (50 to 150 mg daily)	2	5.5

If the 36 lethal cases of lupus nephropathy are grouped according to schedules of treatment, it is found that even here the longest survival was ensured by immunosuppressive therapy. At first, this treatment was confined to steroid-resistant cases of utmost severity. Therefore, though survivals in the series of cytostatic therapy (Table III, groups 3 – 4) have not yet reached those in group 2 where no such therapy has been used, these results must be none the less rated as excellent, since before this therapy the outcome of these cases was hopeless, death inevitably ensuing within a few months. The results might have been still better, had cytostatic treatment been instituted earlier.

Uraemia has remained the prevalent cause of death in lupus nephropathy, at least in the present material (Table IV).

Table V sums up the results in 33 patients with lupus nephropathy being under close follow-up. Antimalarial drugs alone failed to bring benefit. Full remission was obtained on massive-dose long-term steroid therapy in 8 and by steroid + cytostatic therapy, in 11 cases. Though sporadic small-dose steroid therapy too had often a favourable influence on various, even on severe, systemic manifestations of SLE other than nephropathy (group 2), these results are far from those achieved by massive-dose immunosuppressive steroid therapy, and if even this proves inadequate, cytostatics still offer the chance of full remission.

Since 1963 we have prescribed cytostatics in autoimmune nephrosis if 1) the response to steroids was not satisfactory; 2) steroids had to be discontinued owing to secondary effects.

Table IV

Causes of death in the fatal cases of lupus nephropathy

Cause of death	Number of cases
Uraemia	26
Heart disease	8
Apoplexy	1
Septicaemia	1
Total	36

Table V

Patients with lupus nephropathy under follow-up. Treatment and results

Group	Therapy	Full remission	Incomplete remission	Partial effect	No effect	Total
1.	Antimalarial agents	—	—	—	2	2
2.	Corticosteroids, 40 mg daily (sporadic)	1	1	2	3	7
3.	Corticosteroids, 50 mg daily (long-term)	4	4	—	1	9
4.	Corticosteroids, 50 mg daily + cytostatic agents	3 * **	3 * **	—	—	6
5.	Corticosteroids, 60 mg daily + cytostatic agents	* 2 *	* 3 ***	* 2 **	* 2 *	9
	Total	10	11	4	8	33

* 6-MP, 50 to 100 mg daily, by mouth
** Cyclophosphamide, 200 mg daily, by mouth
*** Azathioprine, 50 to 100 mg daily, by mouth

The response to steroids was poorest in patients with chronic glomerulonephritis. The nephrotics responded favourably to steroids but the presence of isosthenuria seemed to contraindicate their use.

As a result of our observations we have adopted the following rules. (a) If massive-dose steroid therapy had failed to bring any favourable change in the renal condition in 4 weeks or (b) if steroids were poorly tolerated, or (c) if there were signs of progression despite steroid therapy, or the condition had been very poor from the very outset (asthenuria, oliguria, increase in NPN),

steroid-treatment was discontinued or reduced to the limits of tolerability and cytostatic therapy was instituted under close supervision of the blood counts.

A moderate leukopenia does not yet contraindicate cytostatic therapy, since this seems to be connected with the autoimmune mechanism of the disease itself and may be often associated with nephropathy of this kind. Paradoxical though it may seem, it is none the less consistent with the autoimmune mechanism that cytostatic treatment may result in a temporary rise of the leukocyte count.

If there is no obstacle to the use of cytostatics in adequate doses, in the nephrosis syndrome a favourable response may be expected within 3 or 4 weeks. The response is dose-related which implicates that there may be a need for high doses carrying serious hazards to haemopoiesis. The vulnerability of the blood-forming system shows wide individual variations. If leukopenia develops, treatment is discontinued for a time and resumed with some other cytostatic drug. If this too causes leukopenia, we have to compromise on the dosage by resuming treatment with tolerated doses after the restitution of the bone marrow.

As to the incidence of spontaneous remissions in SLE, we had a single case in our entire series of 250 patients. The patient had been under treatment for nephrosis of extreme severity 20 years before the detection of SLE at the First Department of Medicine in Budapest and, being given up as hopeless, discharged home where she made a spontaneous recovery in about half a year and remained symptom-free until the mid-fifties. She was then admitted for polysystemic SLE which had made its appearance at that time. After a course of steroid therapy, full remission ensued. The diagnosis of SLE was based on the typical skin manifestations and on the demonstration of the LE-cell phenomenon. A subsequent recurrence, associated this time with proteinuria, though in the presence of normal renal functions, was also found to respond to conventional steroid therapy. Thus, according to our experience, the incidence of spontaneous remission of the typical syndrome in adults is at most 1% (1 in 80).

The autoimmune renal manifestation confined to proteinuria shows wide fluctuations even in the same individual. Isolated proteinuria may persist for long years without developing into nephrosis with hypoproteinaemia and oedema or affecting renal functions. In other cases, persistent proteinuria is associated with a gradual impairment of renal functions while preserving its dry character, i.e. there is no nephrosis-syndrome marked by oedema. Renal lesions associated with isolated proteinuria without oedema hardly ever respond to immunosuppressive therapy, at least not within the usual 3 or 4 weeks in a manner to be attributable to the drug and not to a spontaneous remission.

The various cytostatic drugs seem to act in a different manner on the diverse manifestations of the autoimmune diseases and even on the autoimmune reactions directed at particular tissue constituents. In other words, these drugs might be considered to have individual activity spectra [8]. This makes it our objective to select the appropriate cytostatic for a given type of

autoimmune disease, i.e. the compound eliciting an optimum response in that particular process in doses still harmless to the bone marrow or without any other adverse effect.

For such reasons, in autoimmune nephropathies of the "primary nephrosis" or "Nephritis mit nephrotischem Einschlag" types 6-mercaptopurine (6-MP or azathioprine) should be prescribed first. Failure of the maximum tolerable doses to bring about any convincing improvement in 3 to 6 weeks makes it warrantable to change over to cyclophosphamide or, possibly, to chlorambucil after a treatment-free interval. 6-MP has very little, if any, influence on the various other systemic manifestations of SLE, therefore it should always be given in combination with prednisolone in polysystemic syndromes involving an immune (or autoimmune) genesis.

The major hazards of cytostatic therapy are an irreversible damage to the bone marrow and a loss of resistance to infections. The latter may be brought under control by the use of antibiotics. Intercurrent infection accounted for no fatality in our material. We are not in favour of a preventive administration of antibiotics in immunosuppressive therapy. These agents should be reserved for therapeutic use in the presence of an infective process and should be directed at the infective agent. (Radiological evidence of tuberculosis, even though of "inactive" appearance, requires a similar attitude.) In view of a possible bone marrow injury, the lines of treatment are entirely different from those to be followed in leukaemia. While for instance in myeloid leukaemia a fall of the leukocyte count even below 1000/μl is generally no cause for major concern, in autoimmune diseases the limit of drug-induced leukopenia should never be below 3000/μl. A fall of the leukocyte count to this level requires the suspension of treatment until a count of 3500 to 4000/μl has been surpassed.

The following two cases are illustrative of the effects of cytostatic therapy.

Case 1. K. K., a 60-year-old female, with a long history suggestive of autoimmune disease, was admitted on 13 March, 1962 for typical nephrosis with generalized oedema having made its appearance two months earlier. She was oliguric, isosthenuric, daily average protein excretion was 7 g, serum protein concentration less than 5 g per 100 ml, creatinine clearance 22 ml/min, NPN 77 mg per 100 ml. The only symptom of SLE was a transitory positivity of the LE-cell phenomenon. Despite corticoid therapy she steadily went downhill, with deterioration of the laboratory results. Elevation of the steroid doses failed to halt progression (NPN 112 mg per 100 ml, leukocytes 2200/μl). At this juncture, she was started on 6-MP administered in doses of 50 mg daily, in combination with prednisolone 30 mg daily. The response produced in three weeks was dramatic. Diuresis increased, this was followed by the disappearance of oedema, a rise in GFR and the normalisation of NPN. As a preventive measure, 6-MP was given in the same doses for another 10 weeks. Full remission with freedom from any renal disturbance ensued [6]. Four years later there was a recurrence of nephrosis, proteinuria and oedema having appeared within three weeks in the presence of a fairly good concentration capacity. On being given again 6-MP in doses of 100 mg daily, full remission ensued by the end of the second week. At the last follow-up the patient displayed no abnormality apart from a hepato-splenomegaly which had been present before. Renal functions were normal, the urine contained neither protein, nor casts or other abnormal elements, the specific gravity was 1021 (Fig. 1).

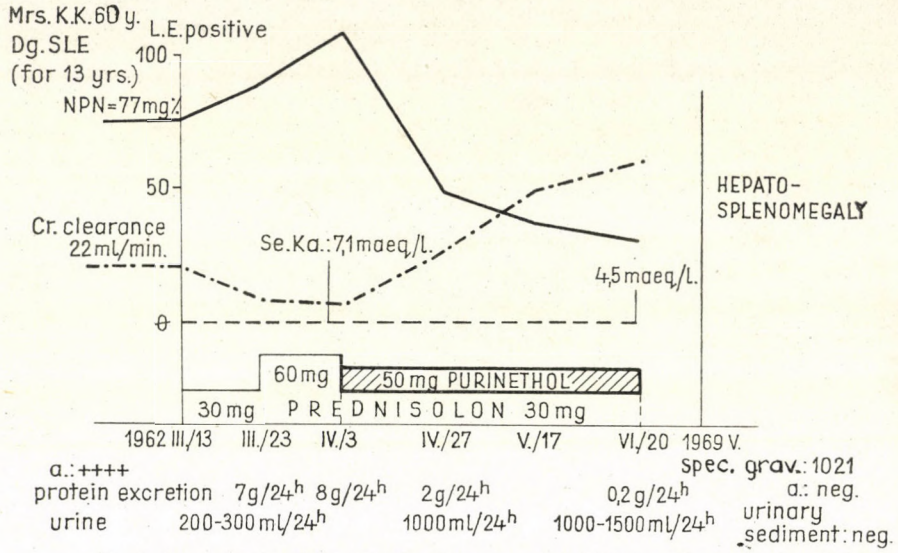

Fig. 1. Case report of Mrs K. K., 60 years old. (NPN = Non-protein N)

The next case proves the informative value of the variations in the complement level and the dose-response relationship of cytostatic therapy (Fig. 2). Having been first seen in 1969, this patient does not belong to the 1953/68 series.

Case 2. M. T., a 24-year-old female, had been suffering from various manifestations of SLE for the last four years. Presenting with fever, pericarditis, arthralgia, purpura, lymphadenitis on an earlier admission, she responded to appropriate therapy with full remission

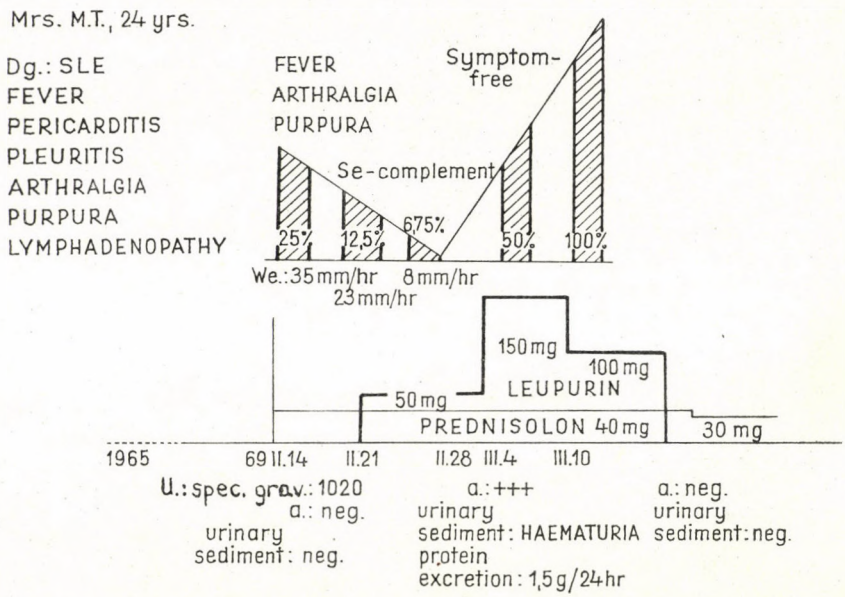

Fig. 2. Case report of Mrs M. T. 24 years old, with SLE. Serum complement level in percentage of the normal value

Readmission on February 14, 1969, for arthralgia, fever and purpura of recent origin. The urine was normal but the serum complement titre attained only 25% of the normal value. The LE-cell phenomenon was negative. On prednisolone treatment the condition improved but the serum complement level continued to decline. Treatment was then combined with 6-MP administered in doses of 50 mg daily. Eight days later a further fall in the serum complement level to 1/16 of the normal values was noted in association with proteinuria, haematuria and oedema. The dose of 6-MP was raised to 150 mg daily, to be reduced to 100 mg upon the first signs of improvement. As a result, the serum complement level attained normal values and the proteinuria and the haematuria disappeared (Fig. 2). This case is particularly illustrative of the informative value of the serum complement level confirmed in other cases, too. A fall in this level provides a signal for the intensification of therapy. Whether the administered amounts of corticoid or those of cytostatic drugs known for their suppressive effect on autoimmune reactions have been inadequate, elevation of their doses over the threshold may still result in a prompt response.

Discussion

General acceptance of the cytostatic agents for immunosuppressive therapy was a slow process, particularly before 1964 [2, 3]. Though, personally, we have been advocating the use of these drugs in autoimmune nephropathy ever since 1962, giving due consideration to their hazards, it was a long time before any therapeutic attempt of this kind was undertaken. This may have been due to the very nature of SLE and to its responsiveness to individual drugs. A considerable time had to elapse before we have recognized that the effects of the various cytostatic agents differ in character and in degree, not only as regards the lesions of different localization but also lesions of different types affecting the same organ. It is, therefore, a mistake to rely on a single agent in the belief that it would act in the same manner on all processes of immunological pathogenesis, regardless of type or localization. For instance, in 1949, we had been prompted by the favourable observations of CHASIS, GOLDRING and BALDWIN [1] with nitrogen mustard in glomerulonephritis to study the effect of mannomustin in SLE; this trial was, however, a failure. Since the acute polysystemic syndrome of SLE, in its extreme diversity, represents a consistent entity, nothing could be more ill-founded than the belief that a single drug, picked out at random from the wide range of cytostatic agents, must necessarily achieve full control of the entire syndrome. Failure of realizing this inevitably ends in disappointment and leads to the view that "cytostatic therapy" (understood in a general sense, no distinction being made) is "merely of academic interest, toxic, and its value has yet to be established" [3]. In the light of our observations, generalizations of this kind are not only erroneous but also harmful.

In recent times, parallel with the spread of renal transplantations, it is being increasingly realized that "immunosuppressive" treatment applied in time and in an adequate manner, may ensure success in autoimmune nephropathy. Thus the use of these drugs should be tried with skill in all immune processes destructive to the kidney so as to avoid the necessity of intermittent haemodialysis or of renal transplantation [9].

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SODIUM AND WATER EXCRETION IN DOGS WITH RENOVASCULAR HYPERTENSION

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Chronic hypertension was induced in dogs by complete ligation of one renal artery; in earlier studies the condition proved suitable for an experimental model of human renovascular hypertension. The present study was concerned with sodium and water excretion in dogs with renovascular hypertension induced in this manner. Under basal conditions, i.e. without loading, the animals exhibited normal fluid and electrolyte excretion, either in the original normotensive or in the induced hypertensive state, during the entire observation period of 360 days. On loading with electrolytes and water excretion significantly diverged from that recorded in the normotensive state. It is suggested that the enhanced production of renin-angiotensin, or rather its direct inhibitory effect on tubular reabsorption, is the primary cause of the increased loss of sodium and water in renovascular hypertension.

In earlier studies (FEKETE, 1967, 1970) the complete constriction of one renal artery with an intact other kidney was shown to be followed by chronic arterial hypertension in the dog. The condition was characterized by a slow deterioration, though no complete failure, of renal function, leading to spontaneous death of the animals within a period of variable length. We have thus been able to produce an experimental model which in respect of its functional and morphological characters may be regarded as representative of renovascular hypertension in man. The model may claim the advantage of allowing to study renovascular hypertension in the dog and, in view of the fact that the kidney with the constricted artery ceases to function in 3 to 4 months, its responses reflect the condition of the "unaffected" contralateral kidney.

It is generally known that renovascular hypertension is associated with disturbances of the fluid and electrolyte metabolism (GROLLMAN and SHAPIRO, 1953; PICKERING, 1968), in particular with 1) an abnormal retention of sodium and water in the tissues; 2) an enhanced excretion of ADH; and 3) an increased sodium and water excretion by the kidney.

The present study has been concerned with some parameters of the fluid and electrolyte balance in renovascular hypertension induced by constriction of one renal artery. During the entire 360-day period of observation the dogs with induced hypertension as compared with their original normotensive state 1) exhibited no major alteration of the basal sodium and water excretion; 2) but did so under the effect of sodium and water loading.

Material and methods

In adult female dogs under pentobarbital anaesthesia and sterile conditions from the paracostal approach the renal artery of one side (if present, also the accessory renal branches of the aorta) was ligated at its origin, the wound was closed, and perineotomy was performed. Mean arterial pressure was measured in the femoral artery with a mercury manometer prior to the intervention and at 20- to 30-day intervals postoperatively, invariably in the alert state of the animals these having been familiarized with the procedure.

1. Basal water and sodium excretion. Urine was collected with a catheter and blood samples were withdrawn from six alert dogs accustomed to the procedure, in the normotensive state prior to ligation and in the hypertensive state consequent upon it, at intervals of 40 days, each time during 2 to 3 successive periods of 20 to 30 min. The animals were fasted before each study for 16 hrs. but were allowed water ad libitum.

2. Water and sodium loading. After the control values had been established, a) tepid tap water was administered by the oral route in an amount corresponding to 3% of body weight; b) a 5% NaCl solution was administered by the intravenous route in an amount corresponding to 1% of body weight. Urine was collected and venous blood samples were withdrawn at 30 minute intervals on 5 occasions (5 times 30 min. = 150 min). The investigations included measurement of hematocrit, endogenous creatinine and sodium concentration in serum and urine. An interval of at least 4 days was observed between the water and salt loading tests. In the course of the 360-day period of observation, a total of 9 water and salt loading tests was performed.

The animals were kept on standard food supplemented by kitchen scraps. Water and sodium intake by the food showed little variations owing to the standard conditions of the diet.

For the calculation of endogenous creatinine clearance, sodium and water excretion, the diuresis per minute was referred to sq.m. body surface derived from body weight. The results were evaluated by the t test (FISHER 1946).

Results

Basal values, i. e. those obtained without loading, are summarized in Table I. As control values, the results of 11 tests performed in 6 dogs were used. In the successive stages of hypertension, the "pathological" basal values have been derived from approximately the same number of animals.

In the course of the observation period of 360 days, blood pressure was significantly ($p < 0.001$) higher, urinary output and sodium excretion were also higher although not significantly different from those of the normotensive controls, owing to the wide scatter of the figures. Endogenous creatinine clearance showed a significant decline during the hypertensive state, serum creatinine and sodium levels remained unchanged.

During the 360-day observation period water loading was followed by water diuresis all throughout, attaining its peak in 60 to 90 min. in both normotensive and hypertensive animals (Fig. 1). The continous heavy line in Fig. 1 shows diuresis in the control condition and the thin lines represent water diuresis registered in the successive stages of hypertension. In the last-named condition, the ascending limbs of the curves tended to be steeper, they were shifted to the left, otherwise their pattern was similar to that of the controls. Beyond the 260th day of hypertension they became flatter, as water excretion was protracted. In Fig. 2, where these relationships are shown, total water

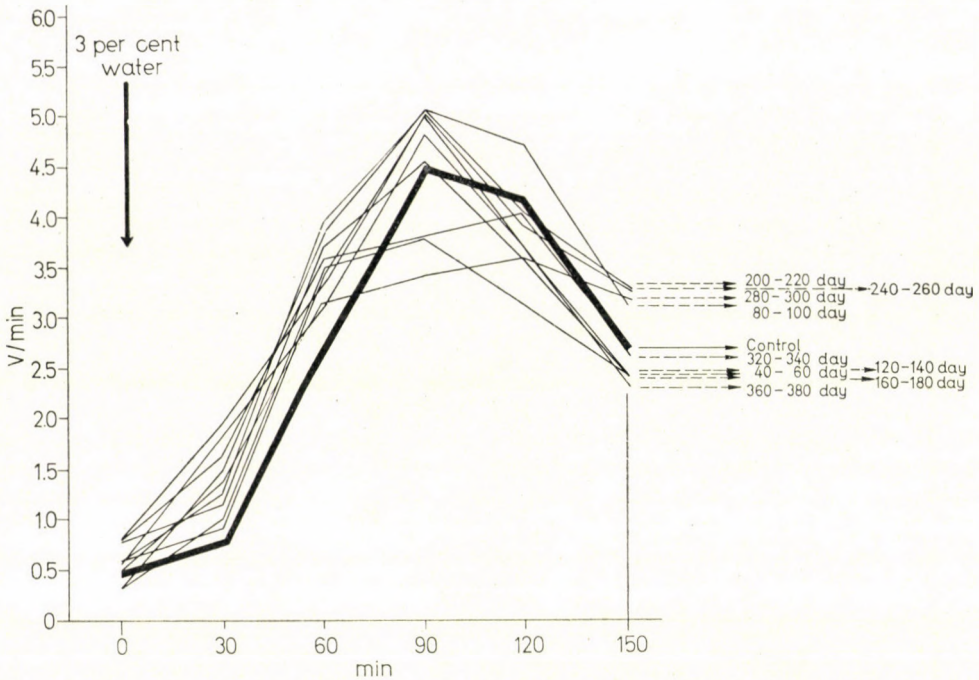

Fig. 1. Water diuresis in six normotensive dogs (control, continuous heavy line) and in the same animals in the successive stages of hypertension (thin lines). The figures beside the graphs refer to the respective day after ligation

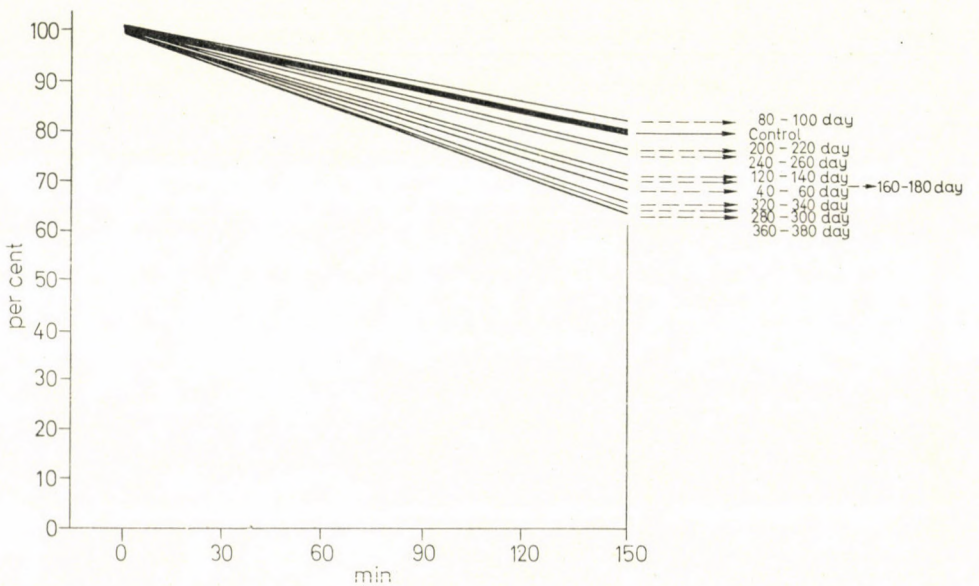

Fig. 2. Water output expressed in per cent of intake. Normotensive controls = continuous heavy line; successive stages of hypertension = thin lines. The figures beside the graphs refer to the respective day after ligation.

Table I

Parameters in six dogs under basal conditions, i. e. without loading,

	number of measurements	blood pressure mmHg	V ml/min	C_{creat} ml/min
normotensive control	11	135±3	0.39±0.06	47±4
hypertensive 40— 60 day	12	175±5	0.58±0.15	45±7
hypertensive 80—100 day	12	180±5	0.65±0.16	45±6
hypertensive 120—140 day	14	175±4	0.68±0.12	34±4
hypertensive 160—180 day	12	175±3	0.73±0.12	41±4
hypertensive 200—220 day	10	170±5	0.50±0.09	41±5
hypertensive 240—260 day	12	175±4	0.67±0.20	39±5
hypertensive 280—300 day	12	170±5	0.69±0.27	40±2
hypertensive 320—340 day	12	170±3	0.85±0.33	37±4
hypertensive 360—380 day	10	170±3	0.34±0.10	35±5

output in 150 min is expressed in per cents of water intake. While in the normotensive state water excretion (heavy line) corresponded to 80% of the intake, in the hypertensive state there was a declining tendency and water output on the 280th day was merely 65% ($p < 0.02$); on the 320th day, 68%; ($p < 0.01$); and on the 360th day, 64% ($p < 0.001$).

Salt loading consisted in the slow intravenous administration of a 5% saline solution (800 to 840 mEq/l) in an amount corresponding to 1% of body weight, totalling approximately 80 to 120 mEq sodium and 100 to 150 ml water. Taking the amount administered as 100%, it was found that 44% of the sodium and 157% of the water administered were excreted within 150 minutes in the normotensive state (control periods), as represented by the heavy

Table II

Renal parameters registered during 150 min after loading with 5% saline in normotensive

	number of measurements	0' control	30'	60'	90'	120'	150'
		normotensive					
C_{creat} ml/min	6	47±8	64±14	67±8	71±7	76±12	84±10
S_{Na} mEq/min	6	150±2	165±4	164±4	161±2	157±3	153±2
F_{load}	6	7.1	10.6	11.0	11.4	11.6	12.9
V ml/min	6	0.39±0.05	2.27±0.84	2.36±0.43	2.56±0.36	2.26±0.42	2.12±0.36
$U_{Na} \cdot V$ μEq/min	6	47±9	452±193	534±153	579±118	586±143	548±116
E/F per cent	6	0.7	4.3	4.9	5.1	5.1	4.3
R per cent	6	99.3	95.7	95.1	94.9	94.9	95.7

in the normotensive state and in the successive stages of induced hypertension ($\bar{x} \pm s_{\bar{x}}$)

S_{Na} mEq/l	$F_{Na\ load}$ mEq/min	$U_{Na} \cdot V$ μEq/min	E/F per cent	R per cent	S_{creat} mg per 100 ml
150±1	7.1	0.047±0.009	0.7	99.3	0.9±0.01
150±1	6.8	0.071±0.029	1.0	99.0	0.9±0.01
153±2	6.9	0.085±0.022	1.3	98.7	1.0±0.01
147±1	5.0	0.068±0.014	1.4	98.6	0.9±0.02
148±1	6.1	0.078±0.013	1.3	98.7	0.9±0.01
150±1	6.2	0.059±0.017	1.0	99.0	0.9±0.01
148±2	5.8	0.075±0.025	1.3	98.7	0.9±0.01
150±2	6.0	0.057±0.018	1.0	99.0	0.9±0.01
152±2	5.3	0.052±0.021	1.0	99.0	0.9±0.01
150±1	5.3	0.050±0.020	0.9	99.1	1.0±0.01

lines in Fig. 3. In the successive stages of hypertension, sodium and water excretion (thin lines) after loading increased steadily deviating significantly from the controls, i. e. corresponding for sodium to 69% ($p < 0.1$) between the 80th and 100th days, to 70% ($p < 0.1$) between 160 and 180 days, to 64% ($p < 0.1$) between 320 and 340 days; the values for water excretion were 234% ($p < 0.02$) between 120 and 140 days, 221% ($p < 0.1$) between 160 and 180 days, 205% ($p < 0.1$) between 320 and 340 days, and 238% ($p < 0.05$) between 360 and 380 days.

The continuous changes consequent upon the administration of a load of 5% saline are shown in Table II. On the left side the mean values and deviations ($\bar{x} \pm s_{\bar{x}}$) relative to six normotensive dogs are seen, and on the right

and hypertensive dogs ($\bar{x} \pm s_{\bar{x}}$)

	number of measurements	0' control	30'	60'	90'	120'	150'
		hypertensive					
C_{creat} ml/min	54	40±6	66±8	61±7	59±7	56±6	56±4
S_{Na} mEq/min	6	150±1	165±5	164±4	161±4	156±4	151±3
F_{load}	54	6.0	11.0	10.2	9.5	8.8	8.5
V ml/min	54	0.68±0.14	3.86±0.53	3.27±0.16	3.31±0.65	2.82±0.73	2.47±0.56
$U_{Na} \cdot V$ μEq/min	54	66±19	828±141	820±131	803±120	680±120	600±103
E/F per cent	54	1.1	7.5	8.1	8.5	7.7	7.1
R per cent	54	98.9	92.5	91.9	91.5	92.3	92.9

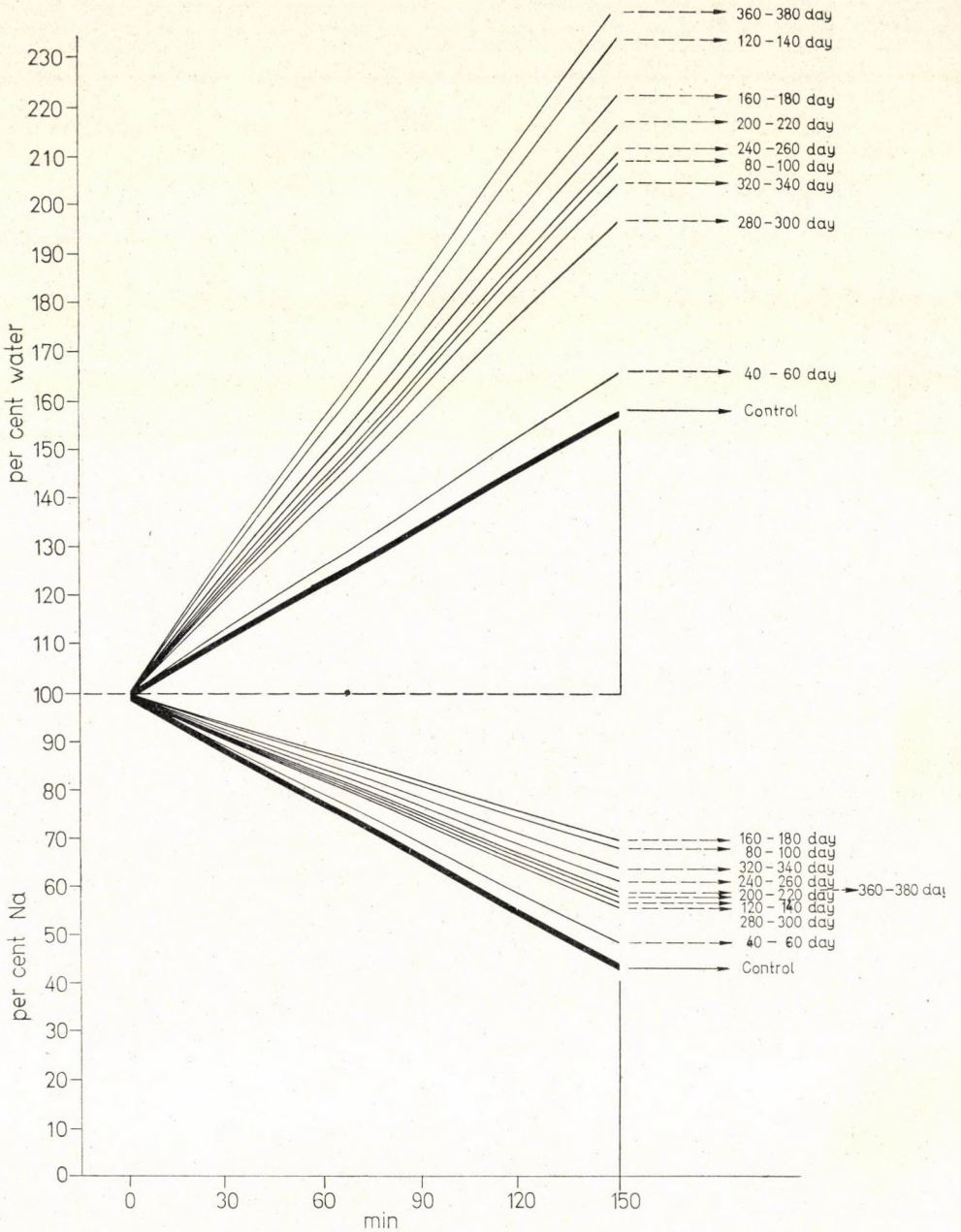

Fig. 3. Sodium excretion after loading with 5% NaCl (lower part), and water excretion (upper part), expressed in per cent of intake. Normotensive values (Control, continuous heavy line; values obtained in the successive stages of hypertension, thin lines). The figures beside the graphs refer to the respective day after ligation

side, the mean values and deviations for the same dogs in the hypertensive state, on 9 occasions altogether. The values for endogenous creatinine clearance, diuresis, and urinary sodium concentration are also shown. The amount of sodium presented for filtration was calculated on the basis of the formula $F_{Na} = C_F \times P_{Na}$, and the amount of sodium excreted with urine, by the formula $E_{Na} = U_{Na} \times V$,

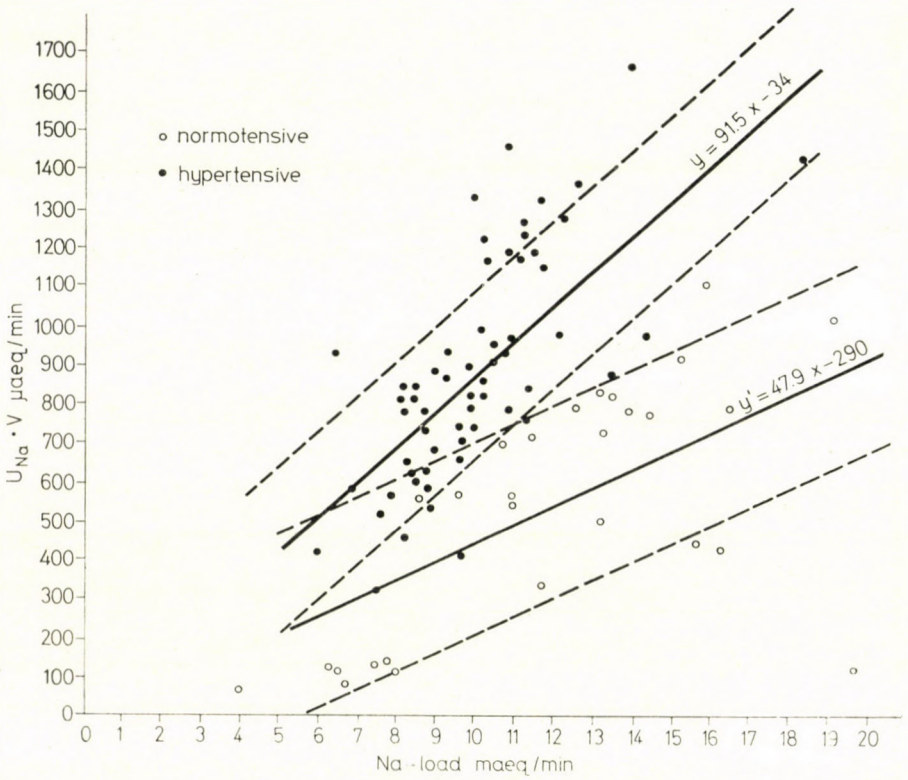

Fig. 4. Sodium excretion plotted against the filtered load in the normotensive (○) and hypertensive (●) state. The regression coefficient of the two lines reveals a significant difference ($p < 0.02$).

tubular sodium excretion and filtration being quantitatively expressed in per cents of the filtered sodium (E/F%, R%). From Table II it emerges that loading with 5% saline was followed by an increase in endogenous creatinine clearance and also in the serum sodium level; in other words, the amount of sodium available for the tubules increased in both the normotensive and in the hypertensive state. However, after identical loadings, urinary sodium excretion was more, i.e. tubular reabsorption less, in the hypertensive than in the normotensive state.

The regression coefficient of the two lines reveals a statistically significant divergence ($p < 0.02$).

Discussion

Renal sodium excretion is the result of an equilibrium, expressed by the equation $C_F \times P_{Na} = T_{Na} + U_{Na} \times V$, where the left side represents the amount of filtered sodium, i. e. the product of serum sodium concentration and of glomerular filtration rate (C_{creat}), and the right side, the total amount of sodium reabsorbed by the tubules (T_{Na}) and excreted with urine ($U_{Na} \times V$). An increase in urinary sodium output may result from an enhanced filtration and/or from a diminished tubular absorption. Owing to methodological reasons, a sharp separation of the parts played by the glomerular and tubular factors is scarcely possible since of the factors of the equation it is only the serum sodium level and the urinary excretion of sodium which can be measured reliably, in contrast to filtration, the measurement of which involves well-known difficulties (BÁLINT 1969).

The occurrence of fluid and electrolyte disturbances in human hypertensive disease, their various clinical types, are amply documented in the literature. The observations are, however, often confusing and the conclusions inconsistent. The available data may be grouped as follows.

According to certain authors (HOLLEY et al. 1951, GROLLMAN and SHAPIRO 1953, DONALD et al. 1958, ALBERT et al. 1958, TOBIAN 1960, etc.) serum sodium concentration increases in experimental hypertension as well as in human hypertensive disease, while according to others (GREENE and SAPIRSTEIN 1952, LEVINE et al. 1961, LEDINGHAM 1953 and others), it remains unaffected.

At the basal level, i.e. without loading, electrolyte and water excretion is not distinctive of a normotensive or of a hypertensive state (BIRCHALL et al. 1953, THOMPSON et al. 1954), basal sodium excretion ranging between 52 and 63 μEq/min in both conditions (EISINGER 1966).

In hypertensive disease, under the effect of electrolyte or water loading, certain disturbances of electrolyte and water excretion become manifest. For instance, as noted by COTTIER et al. (1958), while in normotensive individuals the 3-hr sodium excretion corresponds to 20% of the total infused amount, in hypertensive subjects it is as high as 66%, the 24-hr excretion being 75% in normotensive and 133% in hypertensive individuals. Similar results have been observed with the use of 2.5 or 5% saline solutions by BALDWIN et al. (1958), FARNSWORTH and BARKER (1943), GREEN et al. (1952), THOMPSON et al. (1954), HOLLANDER and JUDSON (1957), HOOBLER et al. (1956), HANENSON et al. (1963), STEIN et al. (1964), etc.

In hypertensive patients, water diuresis is not restricted (BIRCHALL et al. 1953, STAMEY et al. 1961), while ADH excretion is increased (GROLLMAN and SHAPIRO 1953).

On the evidence of the present study, dogs with induced hypertension

exhibit no disturbance of the basal fluid and electrolyte balance. The serum sodium level remains 150 μEq/l in the hypertensive as well as in the normotensive state. Sodium filtration and the excreted and absorbed amount of sodium show no alteration.

From these facts it may be inferred that in the hypertensive state, in the absence of an extra load, the electrolyte and fluid balance is adequate, in other words the organism is capable of adapting the excretion of sodium and water to the basal requirements.

On the evidence of the present findings, dogs rendered hypertensive by ligation of one renal artery exhibit a normal diuretic response to water loading in the early stage of hypertension, and a protracted response in the advanced stage. Loading with hypertonic saline solution resulted in an increase in the filtered amount of sodium in the normotensive and hypertensive states alike, with the difference, however, that in the hypertensive group the increase was followed by an increase of sodium and water diuresis, a finding indicative of a reduced tubular absorption.

On the grounds of these results we feel justified in assuming that the disturbance of the electrolyte and fluid balance associated with hypertension becomes manifest under the conditions involved by loading. In our view these disturbances are due to an impairment of tubular water and sodium absorption, connected with the hypertensive state in which the regulatory mechanisms become inadequate.

Interpretation of renovascular hypertension on the basis of the renin mechanism has been universally accepted. The pressor effect of renin, more precisely of angiotensin, and its inhibitory activity on sodium and water excretion are well established (PICKERING 1968, VANDER 1963). According to GROSS et al. (1965) renin enhances sodium excretion directly. According to LEYSSAC (1964) angiotensin inhibits active sodium-transport in kidney slices. The blocking effect of angiotensin on tubular sodium absorption has been demonstrated by HEALY et al. (1965), a finding confirmed by LOWITZ et al. (1969) and STUMPE et al. (1969) on the evidence of micropuncture studies.

The present model allows renin production by the kidney with a restricted arterial blood supply, since after constriction of the renal artery a collateral system is formed (FEKETE 1967) and provides for 1/10 to 1/5 of the original blood supply of the kidney between the 7th and 110th day after ligation, particularly as concerns the cortical, i.e. the renin-producing areas of the organ. The pressor factor thus produced finds continuous access to the blood stream via the unaffected renal vein. Production of renin by the ischaemic kidney and the presence of the pressor substance in the blood stream after ligation of the renal artery has been shown by bio-assays (FEKETE et al. 1970). Even after total atrophy or elimination of the ischaemic kidney, an increased pressor

activity was demonstrated in the venous blood derived from the intact kidney.

The abnormally enhanced sodium and water excretion under the effect of loading with hypertonic saline solutions in human hypertensive disease as well as in the present model thus seems to be connected primarily with an inhibition of tubular water and sodium transport resulting from the renin-angiotensin activity prevailing in, and characteristic of, renovascular hypertension.

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 28 (1), pp. 119—123 (1971)

AUTOMATIC ANALYSIS OF SPATIAL CARDIAC VECTORS BY THE TRIAXICARDIOMETER

By

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(Received November 30, 1970)

A new instrument, termed triaxicardiometer (TCM), has been devised and a basically new automatic procedure developed for the analysis of the spatial heart vectors. Bioelectrically instructed TCM is an analogue computer yielding absolute magnitudes, azimuth and elevation of the spatial heart vectors directly, without need for further calculations.

The spatial cardiac vectors are defined by their absolute magnitudes and spatial orientation. For this purpose they have to be resolved into components. From vector components X, Y and Z, they may be derived in different ways.

1) Vector electrocardiography [1—7]. Vector components X, Y and Z are represented in the form of ECG-tracings from which the orientation of the vector components is determined, in other words, the angles formed with the frontal, horizontal and sagittal planes and the absolute magnitude of the spatial vectors are computed by vector analysis (Fig. 1).

2) Vector cardiography [4, 5, 8, 9]. Vector components YZ, YX and XZ, i.e. those falling into the sagittal, frontal and horizontal planes are recorded in the form of Lissajous-loops. From the P, QRS and T-loops thus obtained the angles formed with the planes are determined and the absolute magnitude of the spatial vectors, i.e. their plane projection, is computed by vector analysis (Fig. 2).

3) Automatic vector analysis [10, 11, 12]. The absolute magnitude of the heart vectors as well as their spatial orientation defined by the azimuth and the elevation, are shown diagrammatically or numerically by an analogue or digital computer indicating the vector components X, Y and Z, i.e. for the ECG leads X, Y and Z.

In collaboration with Dr. E. Solti eng. we have constructed a new instrument allowing automatic vector analysis [13]. The apparatus, termed triaxicardiometer (TCM), is an electronic analogue computer to be connected as an adapter with any multichannel ECG-equipment or simultaneous recorder (Fig. 3). (The model of the equipment has been executed by the Research Institute of Precision Engineering, Budapest.)

Principle of operation: Biocurrents of the Frank [4] or other lead systems directly related to the components Vx, Vy and Vz of the spatial heart vector instruct the TCM continuously to indicate the absolute magnitude (V) of all the heart vectors as well as their angle of inclination (elevation) (ω) formed with the XZ (horizontal) plane, and the lateral angle (azimuth) of their projection on plane XZ formed with axis X (φ) (Fig. 4).

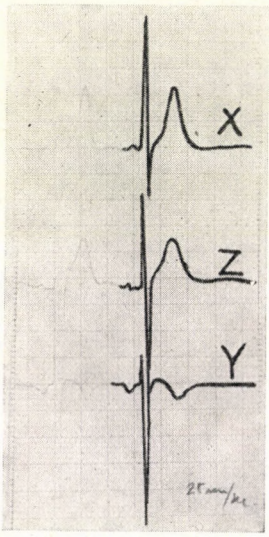

Fig. 1. Vector components X, Y and Z are represented in the form of bipolar lead ECG-tracings

The bioelectrically instructed TCM yields absolute magnitude, azimuth and the elevation of the spatial vector (of all instantaneous vectors) synchronously with the ECG, in particular with standard lead I. In this system the ECG serves only as an indicator for the identification of the spatial heart vectors marked by deflections P, Q, R, ST and T and of their instantaneous vectors. The TCM develops polar coordinates; polar vectors from cartesian coordinates and transforms vector components X, Y and Z into magnitude and polar angles (Fig. 5).

The triaxicardiogram displays ECG standard lead I, the absolute magnitude of the spatial instantaneous vectors (V), azimuth (φ) and the elevation (ω). The azimuth is recorded by two channels, one registers in the range 0° to +180° (φ_1) with an upward, and the other from 0° to −180° (φ_2) with a downward deflection of the direct writing system. The channel for the elevation (ω) registers from 0° to −90° and from 0° to + 90° with an upward and a downward deflection, respectively.

At a paper speed of 50 or 100 mm, a small square of 1 mm represents instantaneous vectors of 10 or 20 msec. In Figure 5, at a paper speed of 50 mm/sec each small square represents instantaneous vectors of 20 msec.

The absolute magnitude and the spatial orientation of the instantaneous vectors may be read directly, independently of the paper speed of the simultaneous recorder. Data are provided by the points at which the perpendicular

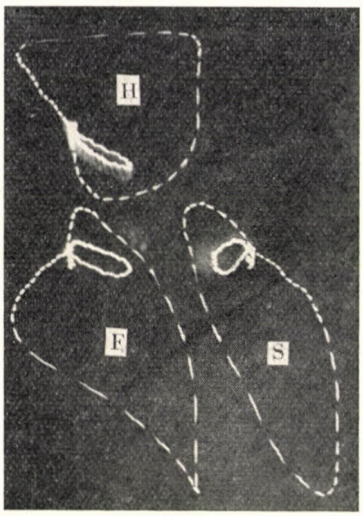

Fig. 2. Vector components X, Y and Z are represented in the form of Lissajous-loops

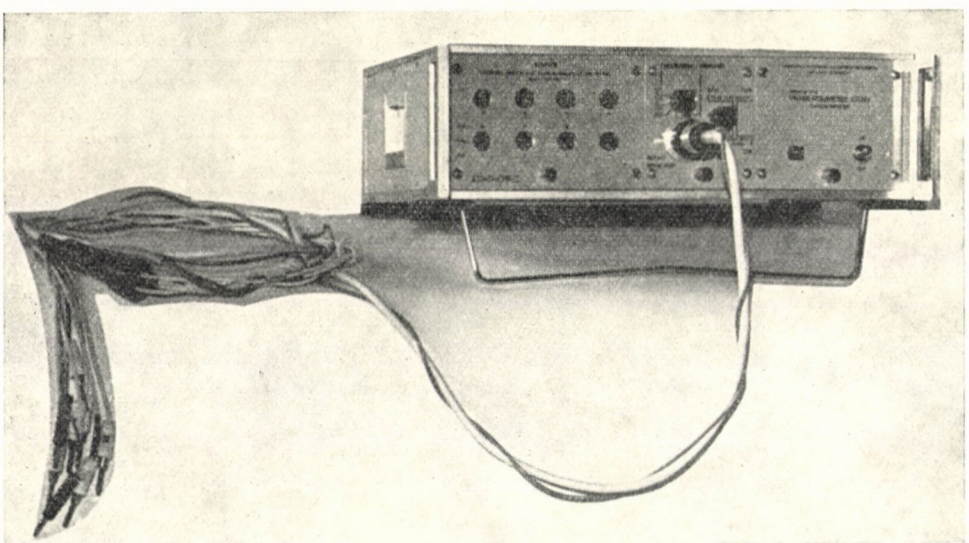

Fig. 3. Triaxicardiometer (TCM) connected with a 6-channel electrocardiograph

lines representing the ECG-deflections, i.e. instantaneous vectors, cut the diagram. For absolute magnitude, V, a small square of 1 mm equals 0.1 mV; for the angles, it equals 6° thus $+6°$ for φ_1 and the downward deflection of ω, and $-6°$ for φ_2 and the upward deflection of ω.

The TCM lends itself to the representation of the vectors X, Y and Z too, in the form of ECG-tracings, offering the following advantages.

1) Cross-checking, as all electric parameters yielded automatically by the TCM can be computed by vector analysis from ECG-leads X, Y and Z.

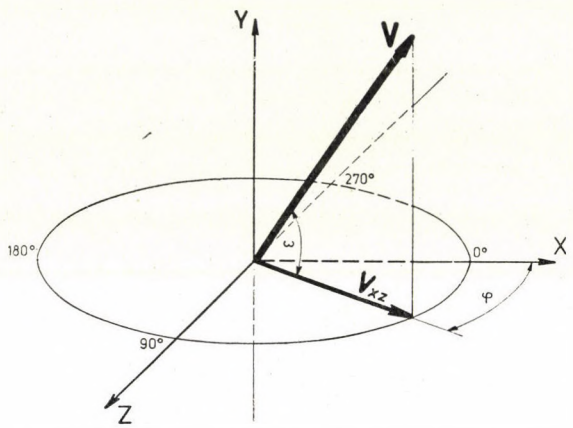

Fig. 4. Absolute magnitude (V), azimuth (φ) and elevation (ω) of the spatial vector

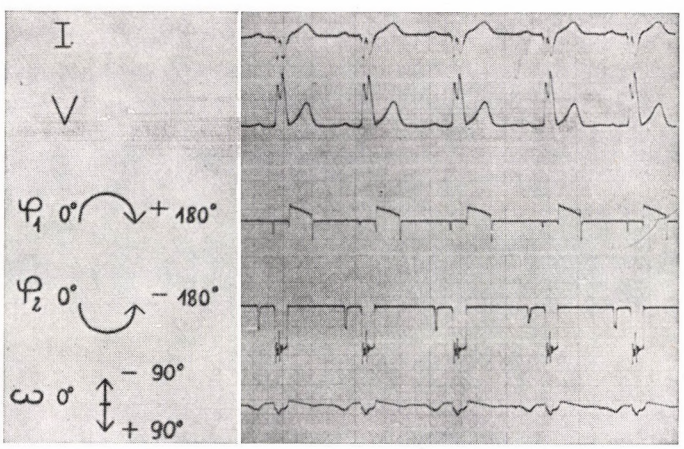

Fig. 5. Triaxicardiogram of a patient with a ventricular septal defect. Spatial vector parameters synchronously recorded with the ECG are the absolute magnitude (V), the azimuth (φ_1 and φ_2) and the elevation (ω)

2) The conventional procedure of defining the spatial vector is coupled with a new automatic method to enable the worker used to conventional 12 or 15 leads [6] to compare the advantages and shortcomings of the two methods, and to confront electric information provided by vector electrocardiography with information gathered by triaxicardiometry.

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RECENSIO

IEE Medical Electronics — Monographs 1—6

Edited by: B. W. WATSON. Peter Peregrinus Ltd. 1971. London. VIII + 248 pp. 30 plates and over 90 illustrations. Price: £5.30; US $ 13.—

This volume is the first of a series published by Peter Peregrinus Ltd. on behalf of the Institution of Electrical Engineers, edited by Dr. Dennis Hill and Dr. Bernard Watson. Each volume in this annual series will contain about six short monographs on topics of electromedical instrumentation and practices.

Volume 1 covers the techniques that have contributed to an increased interest in medical diagnosis. The six monographs are:

— Biomedical applications of electrical-impedance measurements — L. E. Baker, USA
— Indirect methods of blod-pressure measurement — C. A. Greatorex, England
— Application of lasers to medicine — D. W. Hill and T. Powell, England
— Medical telemetry systems — J. Kuiper, Netherlands
— Clinical applications of ultrasonics — P. N. T. Wells, England
— Electromagnetic blood-flow measurements — D. G. Wyatt, England

According to the information from the publisher, the second volume is due in 1972 and will cover topics on electrodes for recording biological signals, Doppler-shift ultrasonic blood-flow techniques, biological amplifiers and evoked responses, and audiometry.

This series will fill a gap in the literature of medical electronics and will be a source of reference for technical departments of hospitals, medical schools and research laboratories. It can be recommended as a useful aid to doctors, life scientists, engineers and physicists.

J. MAJOR

VIth EUROPEAN CONGRESS OF CLINICAL GERONTOLOGY
(8th—11th SEPTEMBER 1971, BERNE)

Preliminary Program

I. Auto-immune diseases in old age

1. Physiopathologie	P. Bordet, Bruxelles
2. Considérations cliniques	G. Cuny, Nancy
3. Communications to the subject (10 minutes)	
4. Conclusions	D. A. Hall, Leeds

II. Drug therapy in old age

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III. Trainability and training in old age

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3. Effort tolerance: Methods of improving Results 4. Risk associated with effort 5. Effects of physical training	K. Lange Andersen, Oslo
6. Practical methods of physical training	W. F. Anderson, Glasgow
7. Physical training and rehabilitation	P. Beckmann, Ohlstadt
8. Group exercises	C. David, Bukarest
9. Motivation for physical training	
10. Psychic consequences of physical training	C. Müller, Lausanne/ Prilly

11. Ideal qualifications for P. T. Instructors
12. Practical results of physical training
13. Conclusions — J. Th. Schreuder and co-worker, Hilversum

IV. Electronic data processing in geriatrics (Computer)

1. Principles and information in geriatrics — A. Colombi, Milano
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3. Information en gérontologie sociale — J. Teillard, Paris

V. Free communications (10 minutes)

VI. Tour of inspection

For informations please apply to:

Prof. B. Steinmann, C. L. Loryhaus, Inselspital
3008 B e r n e (Switzerland)

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ACTA MEDICA

ТОМ 28 — ВЫП. 1

РЕЗЮМЕ

ИЗУЧЕНИЕ АКТИВНОСТИ ЩЕЛОЧНОЙ ФОСФАТАЗЫ В ГРАНУЛОЦИТАХ ПРИ БОЛЕЗНИ ХОДЖКИНА

Ю. БОБРИ

На основе сравнения величин щелочной фосфатазы в гранулоцитах 20 людей с болезнью Ходжкина автор устанавливает, что ухудшение состояния сопряжено с ранним повышением величин щелочной фосфатазы в гранулоцитах, и что сопоставление этих величин с прочими клиническими и лабораторными данными может способствовать раннему распознаванию прогрессирования, рецидивов или обострения процесса.

ОПЕРАЦИОННЫЙ ОПЫТ НА БОЛЬНИЧНОМ МАТЕРИАЛЕ С ИСТИННОЙ ПОЛИЦИТЕМИЕЙ

Д. НАДЬ и Д. ТОМПА

Сообщаются хирургические наблюдения, сделанные у 14 больных с истинной полицитемией и устанавливается, что существенное уменьшение тромбоэмболических и геморрагических осложнений можно обеспечить только достижением стадии ремиссии

ЛИПОЛИТИЧЕСКАЯ АКТИВНОСТЬ АДРЕНАЛИНА НА ПОДКОЖНУЮ ЖИРОВУЮ КЛЕТЧАТКУ БОЛЬНЫХ ОЖИРЕНИЕМ И ЛИЦ С НОРМАЛЬНЫМ ВЕСОМ in vitro

И. ФЕВЕНЬИ, Э. ГОТ и И. КОНЦ

Авторы изучали у 45 лиц с нормальным весом и у 29 больных ожирением самопроизвольный липолиз подкожной жировой клетчатки и липолитическое действие адреналина in vitro.

1. У лиц с нормальным весом и у больных ожирением самопроизвольный липолиз жировой клетчатки оказался одинаковым.
2. Адреналин в обеих группах вызывал одинаковое и достоверное повышение освобождения свободной жирной кислоты.
3. В группе лиц с нормальным весом самопроизвольный и вызванный адреналином липолиз не показал различия у мужчин и у женщин, в то время как у больных ожирением жировая клетчатка мужчин очень мало реагировала на адреналин.
4. У лиц моложе 40 лет жировая клетчатка показала в обеих группах пониженный самопроизвольный липолиз, но более высокую чувствительность к адреналину, чем жировая ткань пожилых лиц, хотя эти различия были достоверными только в группе больных ожирением.

УРОВЕНЬ СЫВОРОТОЧНОГО ГАПТОГЛОБИНА ПРИ АТЕРОСКЛЕРОЗЕ

Ю. СЕКЕЙ, А. ШИМОН и Э. ХОРВАТ

Уровень гаптоглобина был определен в сыворотке 387 больных атеросклерозом (инфаркт миокарда, коронаросклероз, острый цереброваскулярный инсульт, церебросклероз, облитерирующий атеросклероз). Во всех группах величины сывороточного гаптоглобина оказались достоверно повышенными, причем наиболее выраженное повы

шение наблюдалось при острых осложнениях: инфаркте миокарда и остром цереброваскулярном инсульте. Присоединяющийся к атеросклерозу сахарное мочеизнурение не оказало влияния на средний уровень гаптоглобина. Между величинами сывороточного холестерина не наблюдалось корреляции. При еженедельном определении уровня сывороточного гаптоглобина 25 больных инфарктом миокарда максимальное повышение было выявлено к концу третьей недели.

РЕНТГЕНОЛОГИЧЕСКИЕ СИМПТОМЫ СМЕЩЕНИЯ И СДАВЛЕНИЯ ПОЧКИ, ВЫЗВАННЫХ СПЛЕНОМЕГАЛИЕЙ И ГЕПАТОМЕГАЛИЕЙ У БОЛЬНЫХ ИСТИННОЙ ПОЛИЦИТЕМИЕЙ

Д. НАДЬ, Я. ДЬЯРМАТИ и Ч. БАЛАЖ

У 30 больных истинной полицитемией было проведено рентгенологическое исследование почки. Левостороннее смещение почки было выявлено в 28 случаях, а правостороннее смещение в 17 случаях. Размер изменений почек показывает связь со стадией заболевания, а также со степенью выраженности спленомегалии и гепатомегалии. Дислокации, выявленные в стадии обострения, в стадии ремиссии, как правило, уменьшились или отчасти развивались обратно.

КОМПЛЕКСНЫЙ РАДИОЦИРКУЛОГРАФИЧЕСКИ-ОКСИМЕТРИЧЕСКИЙ МЕТОД С ЭРГОМЕТРИЧЕСКОЙ НАГРУЗКОЙ ДЛЯ ОЦЕНКИ РЕАБИЛИТАЦИИ СЕРДЕЧНЫХ БОЛЬНЫХ

М. ХОРВАТ, Т. ДЕБРЕЦИ и К. ЛУДВИГ

В введении к статье авторы описывают методику комплексного радиокардиографически-оксиметрического исследования с нагрузкой, проводимого при катетеризации правой половины сердца. Радиокардиограммы правой половины сердца с изотопом Xe^{133} оказались при повторении хорошо воспроизводимыми, и их изменения при эргометрической нагрузке были соразмерными поведению симультанного разведения метиленовой синьки.

Принимая во внимание рассеяния, в отношении минутного и ударного объемов не было значительного отклонения, а также в изменениях содержания оксигемоглобина в артериальной крови между реакцией нагрузки так называемой нормальной группы (10 чел.) и группы больных с инфарктом двухлетней давности (также 10 чел.). Достойным упоминания является только, что у больных с инфарктом наблюдалось запаздывание повышения частоты и более длительное затихание регионального клиренса Xe^{133}. Благоприятная реакция частоты, сопряженная с более умеренным повышением альвеолярной вентиляции может быть результатом непрерывной реабилитационной терапии с тренировкой, проведенной у больных с инфарктом.

В целях наглядности приводятся также диаграммы нагрузки прочих образцовых случаев: лабильная гипертония с гиперреакцией гемодинамики, хроническое легочное сердце с компенсированным кровообращением, и первичная кардиомиопатия с абсолютной аритмией и субкомпенсированным кровообращением.

ДЕЙСТВИЕ ВИТАМИНА K_3 НА АКТИВНОСТЬ СУКЦИНИЛ-ДЕГИДРОГЕНАЗЫ В КОРКОВОМ СЛОЕ ПОЧКИ ЧЕЛОВЕКА И КРЫСЫ

И. МАРОШВАРИ, Д. ТАНКА и М. КЕЛЛЕР

При помощи гистохимических методов исследования была выявлена активность сукцинил-дегидрогеназы в клубочках, изолированных из коркового слоя почки крысы. Активность клубочкового энзима была определена также количественно. Поведение этих энзимов коркового слоя почки изучалось авторами также под влиянием различных веществ, оказывающих действие на окислительно-восстановительные процессы клеток (нитро-БТ, ТПЦ), а также роль витамина K_3 в окислении янтарной кислоты.

В почках больных нефритом активность сукцинил-дегидрогеназы оказалась пониженной. В противоположность нормальной или нефротической человеческой почке при помощи витамина K_3 не удалось достигнуть существенного повышения активности энзима, особенно в клубочках. Понижение активности энзимов цикла Кребса указывает на тяжелое нарушение обмена веществ в нефритической почке.

РАССЕЧЕНИЕ ПОДТАЛАМИЧЕСКОЙ ОБЛАСТИ ПРИ БОЛЕЗНИ ПАРКИНСОНА И АНАЛИЗ РЕАКЦИЙ, ПОЛУЧЕННЫХ НА ЭЛЕКТРИЧЕСКОЕ РАЗДРАЖЕНИЕ

Й. ХУЛЛАИ

После проведения экспериментов по рассечению бледного шара и таламуса, на основе определенных размышлений, автор выбрал для своих исследований подталамическую область. Для определения цели повреждения он применял новый метод, состоящий в анализе действия предварительно проведенных комбинированных таламо-субталамических повреждений. Он проводил оценку стереотаксических вмешательств, проводившихся в различных структурах, в бледном шаре в O. V. части VL-таламуса и в субталамической области, и нашел, что подталамическое вмешательство даже в случае незначительного повреждения оказывается наиболее эффективным.

Для функционального контроля прицеливания и положения электрода автором были проведены эксперименты по электрическому раздражению. Он расценивал ответы, полученные на электрическое раздражение, проведенное в 135 точках, прежде всего в V. O. участке VL таламуса и в подталамической области. При анализе общей реакции были получены данные, по-видимому подтверждающие взаимодействие, предполагаемое в экстрапирамидных кругах ревербации. На основе подробного анализа автором сделан вывод, что V. O. часть VL таламуса, связи n. Sth. и путей подталамической области, Z. i. и Ra. prl., будучи частями экстрапирамидной системы, участвуют в выработке и автоматизации определенных физиологических и патофизиологических движений, и что экстрапирамидная система является системой, служащей для выработки, автоматизации и организации условий волевых движений, а не двигательной или чувствительной системой.

ГУМОРАЛЬНЫЙ МЕХАНИЗМ РЕЗИСТЕНТНОСТИ ЗАКАЛЕННОГО ОРГАНИЗМА К ЯЗВЕ

Р. ФРЕНКЛ

Сыворотка крови спортсменов, систематически тренирующихся, подвергаясь большой нагрузке, в прежних исследованиях тормозила возникновение экспериментальной язвы у крысы. В настоящих экспериментах под влиянием сыворотки крови тренированных людей у подопытных животных наблюдались следующие изменения:

1. Сыворотка крови тренированных людей вызывала у крыс торможение секреции желудочного сока, стимулированной гистамином.
2. Сыворотки крови тренированных людей понижали у крыс-акцепторов чувствительность к гистамину и серотонину.
3. Сыворотки крови тренированных организмов вызывают торможение реакции гипоталамо-гипофизарно-надпочечниковой системы, наблюдаемой под влиянием напряжения.

Эти явления могут быть частичные факторы противоязвенного действия сыворотки крови.

ВОЗНИКНОВЕНИЕ И РАЗВИТИЕ МИЕЛОИДНЫХ ЭЛЕМЕНТОВ В БЕДРЕННОЙ КОСТИ ЗАРОДЫШЕЙ КРЫС

Б. БУКУЙЯ и А. БАЛАЖ

Авторы изучали ультраструктуру гранулоидных элементов, образующихся в бедренной кости 16—21-дневных зародышей крыс. Ткани фиксировались при помощи глютаральдегида + осмия, окрашивались ацетатом уранила + лимоонокислым свинцом и заливались Дуркупан АСМ.

На 16-ый день эмбрионального развития в бедренной кости наблюдается интенсивное окостенение. На 17—18-ый день между костными перекладинами диафиза образуются межкапиллярно или внутрикапиллярно островки костного мозга. Дифференциация миелоидных элементов происходит очень быстро. На 18-ый день можно выявить уже многочисленные клетки: промиелоцита, миелоцита даже метамиелоциты. Однако эти прекурсоры в отношении структуры отличаются от соответствующих зрелых типов, так как при недифференцированном ядре (краевой гетерохроматин, развитое ядрышко) гранулогенез является менее интенсивным (немногочисленные азурофильные зернышки немного расширенного эндоплазматического остова ядра и цистерна Гольджи). Начиная с 20-ого дня наблюдается уже значительное число совершенно диференцированных гранулоцитов, с перинуклеарным гало, вакуолы dense core вторичная и третичная грануляция.

Наряду с нейтрофильными клетками в бедренной кости сравнительно рано появляются также эозинофильные и базофильные клетки, и хорошо можно наблюдать также эмбриональную дивергенцию эозинофильных и базофильных зернышек, уже в ранней стадии бластулы.

РАССТРОЙСТВА СВЕРТЫВАНИЯ КРОВИ ПРИ ГЛОМЕРУЛОНЕФРИТЕ И НЕФРОТИЧЕСКОМ СИНДРОМЕ

А. ХАМОРИ, Д. БОРОШ, Л. ГОФМАН и И. ПАСТОРИ

Авторы изучали свертывание крови у 70 больных, страдавших острым, 4 больных — подострым и 34 больных — хроническим гломерулонефритом, а также у 20 больных с нефротическим синдромом. Авторы применяли 16 тестов и проводили 780 определений свертываемости крови. Одновременно с этим они следили также за активностью заболевания почек.

В начале типичного острого гломерулонефрита и во время острого ухудшения хронического гломерулонефрита закономерно можно выявить повышение свертываемости крови. Наиболее выраженные признаки повышенной свертываемости крови наблюдались при нефротическом синдроме, будь это синдром идиопатического типа, будь он развился на фоне амилоидоза, диабетического гломерулосклероза или системной красной волчанки. У больных, страдавших синдромом Гудпасчура также авторы выявили повышенную свертываемость крови, несмотря на то, что непосредственно до наступления смерти был выявлен гепариноподобный антитромбин. Понижение свертываемости чаще всего наблюдалось в связи с неактивным хроническим нефритом. Повышенная свертываемость крови является одним из признаков активности нефропатий. Стойкое повышение свертываемости крови означает плохой прогноз.

У восьми больных с нефритом или нефротическим синдромом авторы применяли лечение антикоагулянтами, без достижения резкого улучшения процесса.

ИММУНОСУПРЕССИВНОЕ ЛЕЧЕНИЕ АВТОИММУННЫХ НЕФРОПАТИЙ РЕЗУЛЬТАТЫ 16-ЛЕТНЕГО НАБЛЮДЕНИЯ

Д. ПЕТРАНЬИ, А. ЛЕВИ, Д. СЕГЕДИ, Д. КАКУК и Й. БОБОРИ

Авторы анализируют результаты 16-летнего наблюдения за лечением прототипа автоиммунных нефропатий, люпозных нефропатий. Иммуносупрессивное лечение означает решающий поворот в области прогноза заболеваний почек, которые раньше по большей части были неизлечимыми. Хотя в наши дни больных даже после гибели почек можно сохранить в жизни путем гемодиализа и трансплантации почки, и при помощи иммуносупрессивного лечения можно предотвратить не только отталкивание трансплантата, но возможно также лечение автоиммунных нефропатий. Из 250 больных системной красной волчанкой в течение прошедших 16 лет у 80 больных имелась нефропатия. Судьба 11 из них неизвестна, 30 больных умерли, 33 больных еще находятся под наблюдением авторов. Способ лечения в течение периода наблюдения изменился. В первый пятилетний период (1953—1957 гг.) главной причиной смерти от системной красной волчанки была люпозная нефропатия, так как несистематическая дача небольших доз стероидов и противомалярийная терапия по большей части оказались безэффективными в отношении люпозной нефропатии. В период от 1958 до 1963 года применение интенсивной иммуносупрессивной терапии уменьшило появление нефропатии у больных системной красной волчанкой и улуч-

шило также прогноз больных с нефропатией. В последний период наблюдения (1963—1968 гг.) благодаря добавочному лечению цитостатическими средствами, даже рефрактерные к стероидам больные стали излечимыми. Среди цитостатических средств авторы получали наилучшие результаты после применения меркаптопурина или азатиоприна, которые лучше всего оправдывались также в области предотвращения отталкивания трансплантата. Удовлетворительный результат был получен также после дачи циклофосфамида. По мнению авторов в прогнозе и терапии автоиммунных нефропатий не означает практической разницы возможность актуального выявления фактора LE. Наблюдения авторов в полной мере действительны также для автоиммунных нефропатий без системной красной волчанки.

ВЫДЕЛЕНИЕ НАТРИЯ И ВОДЫ У СОБАК С РЕНОВАСКУЛЯРНОЙ ГИПЕРТОНИЕЙ

А. ФЕКЕТЕ, А. РЕНЬИ-ВАМОШ и А. СИТАШ

У собак посредством полного лигирования почечной артерии на одной стороне было вызвано состояние хронической гипертонии, которое, на основе прежних исследований, можно рассматривать как модельный эксперимент человеческой реноваскулярной гипертонии. Целью настоящих опытов было уяснение условий выделения натрия и воды у собак с гипертонией. Установлено, что без нагрузки, в так наз. базальных условиях, одни и те же животные в течение 360-дневного периода наблюдения, в состоянии нормотонии и в состоянии гипертонии в отношении выделения электролитов и воды не показали существенных отклонений. Однако после нагрузки электролитами и водой реакции животных в состоянии нормотонии значительно отличаются от результатов, полученных в состоянии гипертонии. Авторы придерживаются того мнения, что причинным фактором повышенной потери натрия и воды, наблюдаемой в состоянии гипертонии, является повышение образования ренин-ангиотензина, вернее его непосредственное ингибиторное действие на всасывание в канальцах, выявленное также авторами при реноваскулярной гипертонии.

АВТОМАТИЧЕСКАЯ РЕГИСТРАЦИЯ ПРОСТРАНСТВЕННЫХ ВЕКТОРОВ СЕРДЦА ПРИ ПОМОЩИ ТРИАКСИКАРДИОМЕТРА

З. АНТАЛОЦИ

Для автоматической регистрации пространственных векторов сердца авторы сконструировали новый прибор: триаксикардиометр (ТКМ) и применяли принципиально новую методику. Биоэлектрически управляемый триаксикардиометр представляет собой аналоговую вычислительную машину, непосредственно предоставляющую абсолютные величины, азимуты и угол наклона (возвышение) пространственных векторов сердца, без всякого исчисления.

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THE BUDD—CHIARI SYNDROME

REPORT OF EIGHT CASES

By

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(Received September 21, 1970)

After a brief survey of the Budd—Chiari syndrome, eight cases are reported in four of which the primary process was polycythaemia; in three, tumour; in one case veno-occlusive disease of the liver. In three cases the disease was diagnosed during life. Seven patients died, the eighth is still in remission as a result of therapy directed against polycythaemia.

The first evidence of occlusion of the hepatic vein was reported by Budd [5] in 1845, then the subject was reviewed by Chiari [7] in 1899. Hence the name Budd—Chiari syndrome applied to affections of the liver due to partial or complete obstruction of the hepatic veins. Complete obstruction is associated with the acute form of the syndrome, marked by severe abdominal pain of acute onset, haematemesis, melaena, hepatic coma. Its outcome is fatal, death ensues in a few hours or days [12, 15, 16, 21]. Partial obstruction of the hepatic vein results in the chronic form of the syndrome the essential features of which include hepatomegaly, pain in the region of the liver, slight jaundice, rapid development of ascites, dilatation of the abdomino-thoracic cutaneous collateral veins, massive oedema of the sacral region and of the lower extremities, reduced prothrombin value, increased BSP retention. The outlook is poor in this form, too. The patients live for a few months, survival for a few years is exceptional [6, 7, 15, 16, 21].

To the pioneer observations there is little to add, but knowledge concerning the aetiology, diagnosis and therapy of the syndrome has been increasing.

The underlying cause according to present knowledge includes a congenital, thrombotic or malignant obstruction of the inferior v. cava, obstructive endophlebitis or thrombosis of the hepatic vein, migratory thrombophlebitis or thrombosis, polycythaemia, leukaemia, hepatic abscess, tumour, cirrhosis, gumma, echinococcus, injury, perihepatitis, peritonitis, use of oral contraceptives [5, 7, 12, 15, 16, 20, 21, 26, 27, 36].

The diagnostic advances are largely due to cavography and to splenoportography [10, 18, 19, 36] permitting to locate the obstruction, as also the laparoscopy and needle biopsy providing for a direct visualization of the morphological signs of congestion (nutmeg liver, centrilobular stasis or necrosis, pseudolobulation [7, 12, 25]).

The therapeutic possibilities which are confined to the chronic form of the syndrome are linked with the advances in surgical technique. Splenorenal, portocaval, azygocaval shunts, cavocaval bypass may be life-saving by reducing portal hypertension [10, 18, 36]. The acute syndrome resists all therapeutic attempts. The chances of long-term anticoagulant therapy are limited.

In the light of these considerations it would appear as though, in view of the multiplicity of its possible causative factors, the syndrome was far from rare and, in view of the wide range of diagnostic possibilities, its diagnosis far from difficult. In fact, diagnosed cases are still sporadic [1, 9, 14, 18, 21, 34], the features being generally attributed to cirrhosis of the liver, Banti's syndrome, storage disease, portal thrombosis, etc. [1, 9, 14, 28] and the diagnosis is usually revealed post mortem.

In the period 1963 to 1970 we have observed 8 cases of the syndrome in our Hospital and the two Departments of Medicine and the Institute of Pathology of Debrecen University; these eight cases will be reported below.

Case reports

Case 1. B. L., a five-year-old girl was admitted because of severe abdominal pain of acute onset and meteorism. On admission the abdomen was distended, the liver was palpable 6 to 7 cm below the costal arch, firm and tender on pressure. The urine contained increased amounts of urobilinogen. Quantitative blood counts were normal, the differential count revealed moderate eosinophilia and lymphocytosis. Serum bilirubin was 0.5 mg per 100 ml, thymol turbidity was 2U, gold sol 2U; the total serum protein level and electrophoretic serum protein pattern were normal. Three days later percussion revealed ascites over an area of 4 or 5 cm. Administration of chlorothiazide resulted in the disappearance of ascites and a relief of meteorism. In view of this improvement the parents refused their consent to splenoportography and took the patient. Two weeks later she was brought in because of colicky pains and convulsions. On admission she was unconscious, in a state of shock, ascites was present. Attempts to support the circulation failed and she died 2 hours later. The clinical diagnosis was hepatic coma of unidentified origin. Cirrhosis of the liver?

At necropsy the abdominal cavity contained 300 ml fluid, liver and spleen were considerably enlarged. The cut surface displayed a nutmeg liver pattern. The terminal sections of the hepatic veins in the areas of opening into the inferior v. cava were narrowed barely admitting a blunt probe, their walls were thickened, greyish white in colour. There was a greyish-red clot obstructing the lumen of one of the veins.

Histology revealed general centrilobular congestion, central hepatocellular necrosis, with substantial connective tissue proliferation in the periportal areas and formation of pseudolobuli. The hepatic vein showed fibrous thickening of the media and intima. In the narrowed sections and the minor branches of the hepatic vein distal from this portion there were clots.

Diagnosis: Occlusive endophlebitis of the hepatic veins, Budd—Chiari syndrome (Fig. 1).

Case 2. T. L., a 43-year-old female patient had polycythaemia the beginning of which had been marked by recurrent epistaxis and bleeding of the gums and episodes of crural thrombophlebitis. On admission she displayed a plethoric face, enlargement of the liver by 8 to 9, and of the spleen by 4 to 5 cm. RBC, 5 800 000; WBC, 12 000; platelet count, 400 000. The differential count showed a moderate shift to the left; the bone marrow smear increased erythropoiesis, myelopoiesis, megakaryocytosis. Administration of 4 mCi ^{32}P resulted in clinical and haematological remission with shrinking of the liver and of the spleen by 4 to 5 cm each. Later, however, there was another episode of thrombophlebitis appearing in the right armpit and she was given further 2×100 microCi(!) ^{32}P. Thereupon she became symptom-free and was discharged. The remission lasted three months when she experienced sudden intensive pains in the hepatic region, developed subicterus and became confused. She was admitted and given intravenous corticosteroids, strophanthin, analeptics, but died within three hours. The clinical diagnosis was hepatic coma, polycythaemia vera.

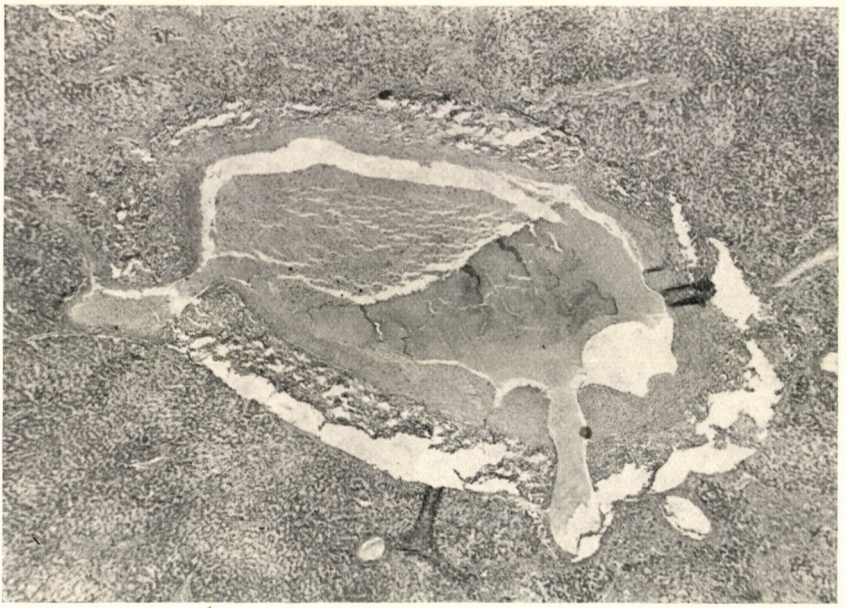

Fig. 1. Major branch of hepatic vein with fibrous thickening and infiltration of its wall by round cells and plasmacytes. The liver shows a nutmeg pattern. H. E. ×40

At necropsy the liver was enlarged, its surface was smooth. The subcapsular regions as well as the cut surface had a mottled appearance with a yellow and brown patchy discoloration, the structure was indistinct. The hepatic veins were filled with firm, dark red adhesive clots.

Diagnosis: Recent thrombosis of the hepatic veins, Budd—Chiari syndrome; polycythaemia vera.

Case 3. Sz. I., a 55-year-old female patient had been complaining of abdominal colics and weakness for the last two days. On admission she was subicteric, she had meteorism, the liver was enlarged by 6 or 7 cm; it was of firm consistency, tender on pressure. There was oedema of the legs. Urinary urobilinogen was increased. RBC, 4 800 000; WBC, 9000; haemoglobin, 15 g per 100 ml. The differential count revealed a slight shift to the left. Serum bilirubin was 2.68 mg per 100 ml, giving a protracted direct diazo reaction, the thymol turbidity and gold sol tests were negative. Serum GOT was 979 U. NPN was 76.5 mg per 100 ml; serum total protein and the electrophoretic pattern were normal. The pains ceased on the third day but ascites developed rapidly and the serum GOT attained 2400 U. On intensive diuretic treatment the ascites practically disappeared. On the 54th day she was discharged with the diagnosis of hepatic cirrhosis but had to be readmitted soon afterwards because of the reappearance of ascites and pains. This time the investigations were started with laparoscopy. After withdrawal of 5000 ml ascites fluid, a liver of uneven, brownish-red surface, enlarged by 5 cm, as well as the normal-sized spleen, could be visualized. The finding was indicative of hepatic cirrhosis. The blood counts were largely the same as on the first occasion, only BSP retention rose to 94%. The ascites was rapidly progressing, the abdominal pains were continuous. Thrombophlebitis appeared on the right lower extremity involving the entire area between the malleolar and the inguinal regions. On repeated abdominal paracentesis, diuretics and the treatment of thrombophlebitis produced symptomatic improvement and the patient was discharged. She was brought back five days later with incipient hepatic coma. The blood counts now were typical of polycythaemia, with RBC, 5 700 000; haemoglobin, 21.3 g per 100 ml; haematocrit, 70%; WBC, 14 000. Despite infusions, glutamic acid, neomycin, cardiac drugs, the patient died seven days later. In spite of the terminal polycythaemia, true interpretation of the signs was missed and the clinical diagnosis remained hepatic cirrhosis.

At post mortem the abdominal cavity contained 9000 ml of fluid. The liver was enlarged, its cut surface displayed a nutmeg pattern. There was a palm-sized greyish-yellow area ad-

jacent to the main trunk of the hepatic vein the lumen of which as well as that of its branches was filled with stratified clots. The portal, lienal and the superior mesenteric veins contained red clots. Both femoral veins were filled with organized clots. There was a considerable hyperaemia of all organs.

Histology: the pattern was that of a nutmeg liver marked by centrilobular hepatocellular atrophy, necrosis, haemorrhages. The portal areas and the lobular centres were connected by fibrous strands, creating a pseudolobular pattern. The branches of the hepatic vein contained thrombi in successive stages of organization. There was fresh clot formation in the branches of the portal vein.

Diagnosis: Thrombosis of the hepatic vein in the stage of organization. Congestive, indurated nutmeg liver turning into cirrhosis. Fresh thrombosis of the portal, lienal and superior mesenteric veins. Organizing clot in the femoral vein. Polycythaemia. Budd—Chiari syndrome (Fig. 2).

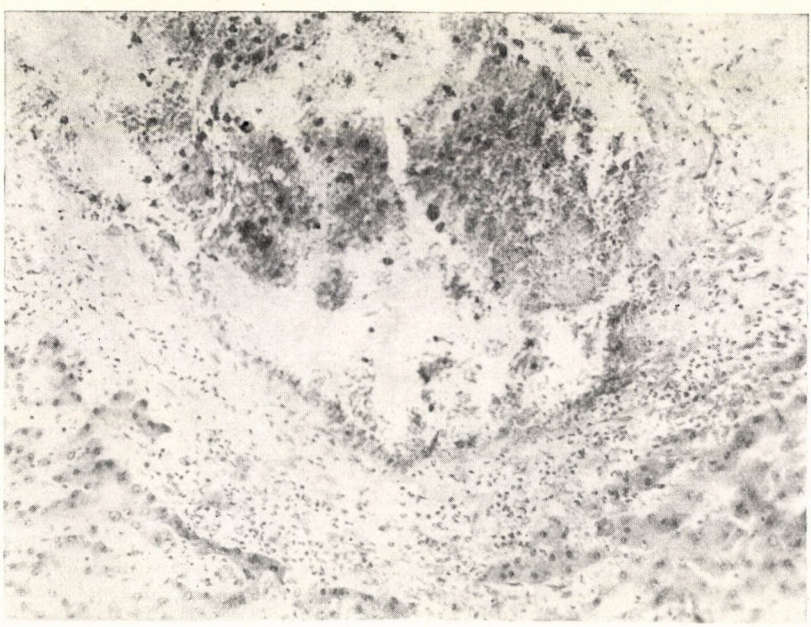

Fig. 2. Sublobular hepatic vein. Oedematous wall with infiltration by granulocytes, lymphocytes, plasma cells and eosinophils. In the lumen a fresh red blood clot. H. E., ×76

Case 4. M. J., a 41-year-old male patient was a chronic alcohol addict who had been under our care on repeated occasions for ascites and oedema. The signs and symptoms had been attributed to cirrhosis of the liver. Cardiacs and diuretics proved beneficial each time. RBC, WBC and platelet counts had been at the upper limit of the normal on these occasions. Readmission was made necessary by severe colics. On admission moderate jaundice, ascites, engorged veins of the abdominal wall were found. The liver exceeded the costal margin by 10 cm, the spleen by 2.5 cm. The lower extremities and the sacral region were oedematous. Serum bilirubin was 2.62 mg per 100 ml, giving a direct diazo reaction; thymol turbidity, 2.65 U; serum GOT, 54 U; prothrombin index, 45%, serum total protein 6.3 g per 100 ml. The results of haematologic investigations were indicative of polycythaemia: RBC, 6 400 000; WBC, 10 000; platelet count, 400 000; haemoglobin, 20 g per 100 ml; haematocrit, 60%; the differential count showed a shift to the left; the sternal bone marrow, increased erythro-, myelo- and thrombopoiesis. On these grounds, Budd—Chiari's syndrome was diagnosed. Administration of 5 mCi ^{32}P and of heparin intravenously was followed by a rapid haematological response but was unable to arrest the progress of the hepatic lesion and the patient died with hepatic coma in the second week of the haematological remission.

At necropsy, the abdominal cavity contained large amounts of fluid. The right lobe of the liver was enlarged, its structure was fibro-nodular. The hepatic veins, the inferior v. cava and the common iliac vein were obstructed by greyish-red clots. The medullary cavity of the right femur revealed bright red bone marrow of unusual extent.

Histology: the lumen of the small and medium-sized branches of the hepatic vein was occupied by blood clots in various stages of organization. The parenchyma exhibited necrotic areas of variable size and appeared to be divided up by coarse collagenous tissue. Bone marrow smears taken from the right femur revealed a hyperplasia of all elements.

Diagnosis: Thrombosis of the hepatic veins in the stage of organization. Fresh focal necrosis and postnecrotic cirrhosis of the liver. Polycythaemia vera. Budd—Chiari syndrome.

Case 5. The 28-year-old female patient K. I. had been referred to us for epigastric pains of which she had been complaining for the last year and which had aroused the suspicion of hepatic cirrhosis. On admission, facial telangiectasia and engorged cutaneous veins in the region of the xiphoid process were noted. The liver was greatly enlarged and of cartilaginous consistency. The laboratory findings were indicative of diffuse hepatocellular lesion. Thymol turbidity was +++; gold sol, 4 U; serum GOT, 54 U; serum aldolase, 9.3 U; BSP-retention, 32%; prothrombin index, 48%. Urobilinogenuria was greatly increased. All this was suggestive of portal cirrhosis, but the history, the constant pains and the laparoscopy findings ruled out the presence of hepatic cirrhosis. At laparoscopy Budd—Chiari's syndrome was not identified: on the considerably vascularized surface of the excessively enlarged liver a normal structure was found to alternate with translucent yellowish prominent areas of patchy character measuring 25—30 mm in diameter. The finding was compatible with a polycystic liver, multiple benign hepatic tumours as well as with haemangioma. Despite a liver-supporting regimen,the patient developed hepatocellular jaundice with slight ascites and enlargement of the spleen by 2 cm as a sign of progression of the process. It was finally a laparotomy which threw light on the true condition. The abdomen was opened from upper midline incision. The veins of the skin, subcutaneous fatty tissue and peritoneum were distended. The liver was grossly enlarged, of smooth surface, brownish-red in colour. No node or cyst was seen. There was some fluid in the abdominal cavity. A biopsy specimen was excised.

Histology: the central veins were dilated, their walls showed hyaline thickening. The parenchyma was marked by centrilobular hypoxaemic necrosis and hyaline hyperplasia of connective tissue. The sinuses were dilated as a sign of congestion. Formation of pseudolobuli was distinct (Fig. 3). The diagnosis was Budd—Chiari syndrome the primary cause of which had yet to be identified. The plethoric face of the patient was suggestive of polycythaemia although the results of earlier haematological investigations had been normal. This time, however, the findings were conclusive, RBC being 5 400 000; WBC, 9000; haemoglobin, 18 g; the platelet count, 380 000. Bone-marrow biopsy was typical of polycythaemia. In the loose medullary substrate the fatty tissue was substituted by cellular elements. Hyperplasia of the erythro- and myelopoietic elements with an increased number of megakaryocytes was noted. Venipunctures and intravenous administration of 6 mCi ^{32}P resulted in a complete haematological remission by the end of two months, parallel with a remission of the Budd—Chiari syndrome. RBC was 4 600 000; WBC, 8000; platelet count, 240 000, haematocrit was 45%, the differential count was normal, the pains had subsided, there was a regression of ascites and of collateral venous stasis, the liver had diminished by approximately 6 cm, the spleen was no longer palpable, BSP retention was 5%, serum GOT, 40 U. It was assumed that, together with the haematological remission, the resulting Budd—Chiari syndrome had also been brought under definitive control. This was, however, not the case. One year later, in the presence of normal blood counts, the signs of hepatic veno-occlusion reappeared, the pains in the hepatic region increased in severity, enlargement of the liver by 4 or 5 cm was noted, BSP retention attained 30%, serum GOT rose to 140 U. This recurrence was, however, followed by a gradual improvement, probably due to a spontaneous recanalisation of the veins. On presenting for a follow-up she reported to be well, the blood and sternal marrow counts were normal, there was no sign of portal hypertension. The liver exceeded the costal arch by 4 cm. BSP retention was 5%, serum GOT, 36 U. She had been well ever since and able to attend to her former activities.

Case 6. The 49-year-old male patient S. K. had been referred to the infection unit because of suspected infectious hepatitis. During the first two weeks the clinical course seemed to confirm the suspicion. There was slight jaundice, the liver was enlarged by 4 cm, the urine contained increased amounts of urobilinogen and bilirubin. Serum bilirubin was 4.26 to 5.48 mg per 100 ml giving a direct reaction. Thymol turbidity was +++; gold sol, 3 U; serum alkaline phosphatase, 8 PhU; ESR, 7 mm/h; RBC, 4 000 000; WBC, 5200. At the end of the second week, sudden severe abdominal pain accompanied by haematemesis and melaena appeared, this was followed by the development of ascites. On these grounds, the possibility of hepatitis was rejected, the signs were attributed to hepatic cirrhosis and the patient was trans-

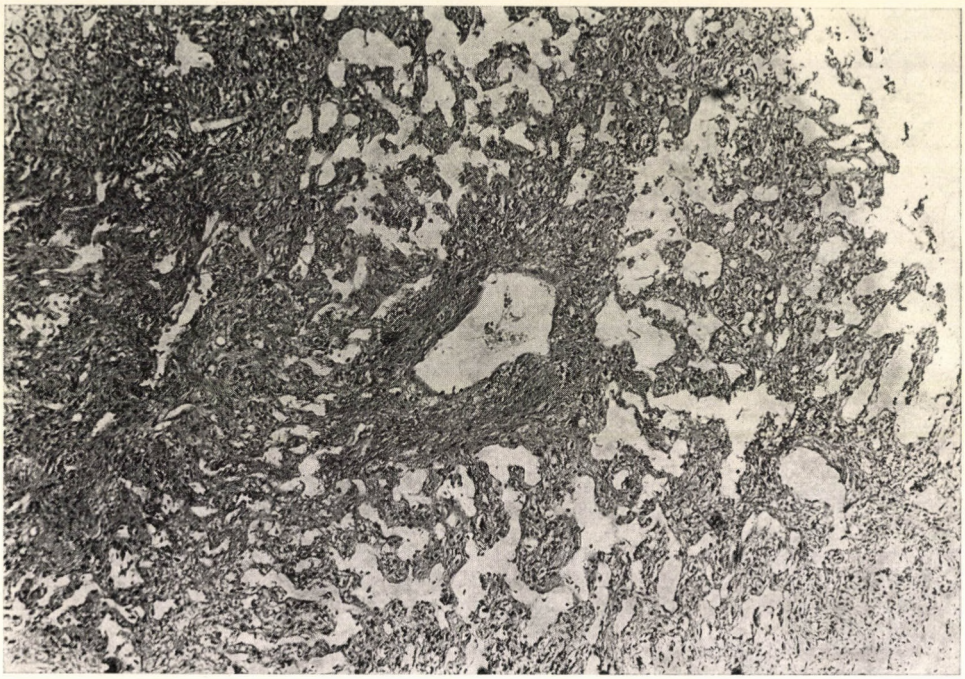

Fig. 3. Fibrosis, hepatocellular atrophy in the centrilobular area contiguous to the central vein. Centrilobular stasis is indicated by the distended sinuses. H. E. ×80

ferred to the medical ward where jaundice and ascites increased rapidly, and the abdominal pains as well as the episodes of haematemesis and melaena appeared more and more frequently. Despite transfusions, haemostatic measures and intravenous corticosteroids the patient died with hepatic coma at the end of the third week. The clinical diagnosis was cirrhosis of the liver of unidentified origin, ruptured oesophageal varices. Hepatic coma.

Necropsy: The abdominal cavity contained 2500 ml fluid. The liver was enlarged and of nodular structure. The right lobe enclosed a fist-sized greenish-brown tumour mass which, 30 mm from the inferior v. cava had ruptured into one of the major branches of the hepatic vein, filling out its lumen. In the branches of the hepatic vein toward the centre there were stratified greyish-white clots. The oesophagus exhibited varicous veins and there were erosions on the gastric mucosa.

Diagnosis: Portal cirrhosis of the liver. Hepatocellular carcinoma of the right hepatic lobe vein with propagation in the vascular lumen and recent thrombosis of the hepatic vein. Budd—Chiari syndrome (Fig. 4).

Case 7. The 69-year-old male patient K. A. had been experiencing persistent epigastric pain and rapid loss of weight for the last month and was admitted in a poor condition. He was moderately jaundiced, the epigastric region was tender on pressure, the liver was enlarged by approximately 8 cm, and of uneven, firm consistency. Uribilinogenuria was marked. BSR was 20 mm/h; RBC, 3 850 000; haemoglobin, 9.1 g per 100 ml, WBC, 10 000. Serum bilirubin was 2 mg per 100 ml, giving a protracted direct diazo reaction, the thymol turbidity and gold sol reactions were negative. Blood sugar was 125 mg per 100 ml. The signs were suggestive of intra-abdominal malignant disease, but in view of the patient's poor condition no such investigations were made. On the fourth day melaena and ascites appeared and the patient became confused. Transfusions of blood, infusions of dextrose, intravenous corticosteroids were administered but he died with hepatic coma on the sixth day. The clinical diagnosis was tumour of the liver with thrombosis of the hepatic veins. Hepatic coma. Budd—Chiari syndrome.

Necropsy: the abdominal cavity contained 1500 ml fluid. The liver was markedly enlarged, of uneven surface, with greyish nodular structures. The right main branch of the

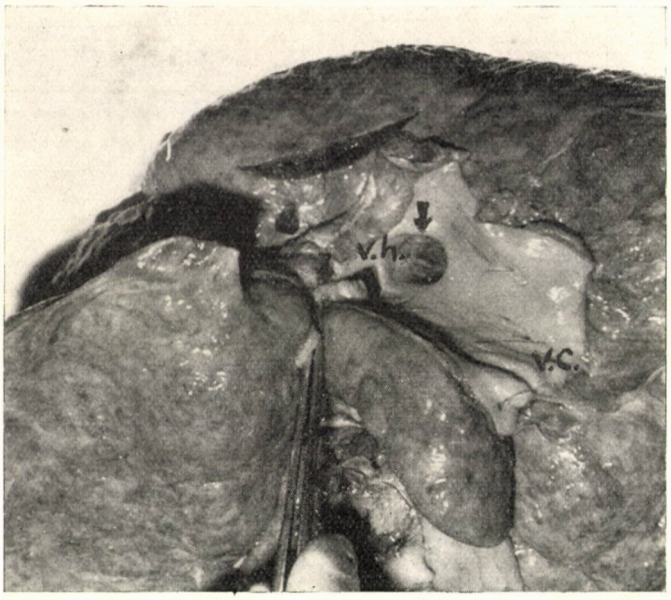

Fig. 4. Hepatic cirrhosis of portal type. The arrow indicates tumour tissue at orifice of hepatic vein (v.h.) as a result of invasion of the vessels by the tumour. Inferior vena cava (v.c.)

hepatic vein was obstructed by a greyish-red stratified blood clot, the cut surface exhibited a palm-sized bright yellow area in the adjacent region.

Histology: the wall of the stomach was infiltrated by an ulcerating tumour made up of atypical glandular elements, solid cellular foci and a fibrous stroma with inflammatory signs. There were metastatic deposits in the liver. The lumen of the hepatic veins was packed with malignant tissue and fresh, stratified blood clots, their walls showed acute inflammation. Malignant emboli were demonstrable in the lumen of the portal vein. The parenchyma of the liver displayed haemorrhagic and necrotic areas.

Diagnosis: Gastric carcinoma with deposits in the liver. Thrombosis of the portal and hepatic veins in consequence of the spread of the tumour. Budd—Chiari syndrome.

Case 8. The 65-year-old male, K. S., a chronic alcohol addict, was admitted in a poor condition. He had been lacking appetite and lost 20 kg in weight in the last six months. He had been jaundiced for a few weeks and had frequent motions of almost black colour. On admission he was pale, emaciated, moderately jaundiced and slightly confused. His mucous membranes were pale, there was sacral oedema. The edge of the liver was firm and sharp, attaining the iliac crest. The urine contained increased amounts of urobilinogen and bilirubin ++; ESR was 70/h; RBC, 2 900 000; WBC, 12 900; serum bilirubin, 4.88 mg per 100 ml, giving a protracted direct diazo reaction; thymol turbidity test, ++++; gold sol, 4 U; serum GOT, 510 U; serum aldolase, 49 U; alkaline phosphatase, 17 Ph.U.; NPN, 60 mg per 100 ml; total protein, 6.67/g per 100 ml, with gamma globulin 42%; the prothrombin index was 78%. Weber's reaction for occult blood in the faeces was strongly positive. The signs were consistent with hepatic cirrhosis of alcoholic origin or with malignant disease of the liver. Laparoscopy failed to confirm either of these possibilities; the liver was grossly enlarged, oedematous, its smooth surface exhibited patches yellow and brown in colour. The abdominal cavity contained some fluid, the veins of the abdominal wall were engorged. The finding was suggestive of hepatitis or of hepatic oedema. Infusions of dextrose, corticosteroids, antibiotic therapy were given but the condition was deteriorating, he became more and more confused and he died with hepatic coma on the sixth day.

Necropsy: the abdominal cavity contained 2500 ml fluid. The liver was enlarged as a result of malignant deposits varying between apple and walnut size. The hepatic veins of the right lobe were obstructed by tumour tissue and red clots. The parenchyma of the liver showed

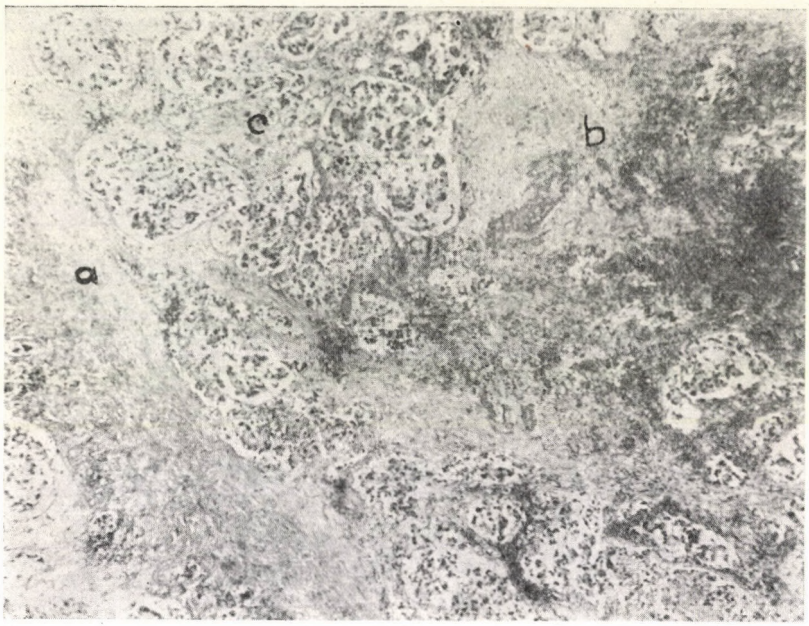

Fig. 5. Major branch of hepatic vein containing metastatic tumour tissue and fresh blood clots. The wall shows signs of reactive inflammation. (*a* = wall of hepatic vein, *b* = fresh blood clot, *c* = tumour tissue). H. E. ×76

fatty degeneration, in the haemorrhagic infarctions were seen. On the major curvature of the stomach at the junction of the corpus and the cardia, a palm-sized area of exulcerated malignant tissue with elevated edges was found.

Histology: solid, anaplastic, adenomatous gastric carcinoma with deposits in the liver The tumour had invaded the right lobar branches of the hepatic vein, infiltrated their walls and extended into their lumen thus producing fresh blood clots. The liver tissue was marked by fatty dystrophy and exhibited extensive areas of haemorrhagic necrosis.

Diagnosis: Gastric carcinoma with secondary deposits in the liver and propagation into the lumen of the hepatic veins with recent thrombosis. Budd—Chiari syndrome.

Discussion

I. The Budd—Chiari syndrome in Hungary

The syndrome is unfrequent in Hungary, as reflected by the small number of reported cases. In accordance with this fact, we found no more than 8 cases in the overall material of our institutes from 1963 to 1970.

The essential data relative to the cases published in Hungarian literature have been summed up in Table I.

The age-distribution between 2 and 69 years was fairly even.

The female-to-male ratio corresponded to 17 : 8.

Table I

Data relative to the cases published in Hungary including those of the present study

m = male; f = female; e.h.o. = obliterating hepatic endophlebitis; cong. h.o. — congenital hepatic vein obstruction; cong. sy. = congenital syphilis; ae.u. = aetiology unknown: p.v. = polycythaemia vera; i.v. = diagnosed during life; p.m. = recognized post mortem; ? = no clinical diagnosis given; Banti's = Banti's syndrome; cirrh. = hepatic cirrhosis; hep. tu. = hepatic tumour; p.s. = observation reported in present study

No.	Age, years	Sex	Aetiology	Diagnosis	False diagnosis	Year of publication	Reference number
1	2	m	e.h.o. (cong. h.o.)	p.m.	chr. hep.	1906	4
2	18	f	e.h.o. (cong. syph.)	p.m.	?	1927	11
3	2	f	e.h.o. (ae.u.)	p.m.	Banti's disease	1938	32
4	38	f	e.h.o. (ae.u.)	i.v.	—	1956	16
5	50	f	e.h.o. (ae.u.)	p.m.	hep. tu.	1957	31
6	45	f	e.h.o. (ae.u.)	p.m.	cirrh.	1958	13
7	67	m	p.v.	p.m.	card. fail.	1958	13
8	68	f	hep. tu.	i.v.	—	1961	29
9	60	f	p.v.	i.v.	—	1961	29
10	61	m	e.h.o. (ae.u.)	p.m.	cirrh.	1962	35
11	31	f	e.h.o. (ae.u.)	p.m.	cirrh.	1964	28
12	49	f	p.v.	i.v.	—	1964	33
13	40	f	e.h.o. (ae.u.)	p.m.	portocav. thromb.	1968	1
14	13	f	e.h.o. (ae.u.)	p.m.	Banti's s.	1968	14
15	56	m	hep. tu.	p.m.	cirrh., hep. tu.	1968	14
16	46	f	p.v.	i.v.	—	1969	34
17	2	f	e.h.o. (ae.u.)	p.m.	storage disease	1970	9
18	5	f	e.h.o. (ae.u.)	p.m.	cirrh.	1971	p.s.
19	43	f	p.v.	p.m.	cirrh.	1971	p.s.
20	55	f	p.v.	p.m.	cirrh.	1971	p.s.
21	41	m	p.v.	i.v.	—	1971	p.s.
22	28	f	p.v.	i.v.	—	1971	p.s.
23	49	m	hep. tu.	p.m.	cirrh.	1971	p.s.
24	69	m	hep. tu. (sec.)	i.v.	—	1971	p.s.
25	65	m	hep. tu. (sec.)	p.m.	cirrh.	1971	p.s.

Aetiologically, the material falls into three groups, *viz.* 1. obstructive hepatic endophlebitis, 12 cases (unknown origin 10, congenital obstruction of the hepatic veins 1, attributed to congenital syphilis, 1); 2. polycythaemia vera 8; 3. malignant tumour of the liver 5 cases.

It is interesting to note that for instance in Egypt and Jamaica obstructive hepatic endophlebitis attributed to nutritional deficiency [3, 22] and in Japan congenital obstruction of the inferior v. cava [36] is the prevalent primary factor of the Budd—Chiari syndrome.

The ratio of in-vivo to post-mortem diagnoses corresponded to 8 : 17.

The majority of misdiagnosed cases had been attributed to cirrhosis of the liver (9 cases).

At the time of publication there are no more than three survivors of all 25 patients.

II. Analysis of the present cases

Aetiology. The aetiologic factor was polycythaemia vera in four, malignant disease of the liver in three, and obstructive hepatic endophlebitis of unknown origin in one of the eight cases.

The hypercoagulability of blood in polycythaemia vera accounts for the involvement of this disease in Budd—Chiari's syndrome. These relationships have important diagnostic as well as therapeutic implications: portal hypertension appearing in polycythaemia is suggestive of the syndrome while successful therapy of polycythaemia vera may result in a remission of the syndrome. From the data on the relationship of the two syndromes [6, 10, 13, 17, 18, 26, 29, 33, 34] as well as from our own observations, it emerges that Budd—Chiari's syndrome is a frequent complication of polycythaemia vera. This is, however, at variance with the statistical figures of PARKER [21] according to which up to 1959 there have been no more than 17 reported cases in which polycythaemia vera had been responsible for occlusion of the hepatic veins. Moreover, CHIEVITZ and THIEDE [8] have followed up 250, and TCHERBAK [30] 219 patients with polycythaemia vera for long years without noting the occurrence of Budd—Chiari's syndrome in a single instance.

Primary and secondary tumours of the liver may induce the Budd—Chiari syndrome by invading the lumen of the hepatic veins or by their compression. The outcome is invariably fatal and it is mostly post mortem that the condition is recognized [12, 13, 14, 20, 21, 25, 29].

The aetiology of hepatic endophlebitis is still problematic. Syphilis, originally incriminated by CHIARI [7] is no longer considered a responsible factor, on the other hand, various other conditions such as alimentary toxaemia, consumption of bush-tea [2, 3], poisoning with Senecio Jacobea alkaloid [23, 24] are of no general validity. The process has no causal therapy, its prognosis is poor.

Diagnosis. Diagnosis has been made during life in 3 of the 8 present cases, in Case 5 on the evidence of laparoscopy combined with liver biopsy, and in Cases 4 and 7 on the basis of the clinical picture. This proportion is, on the whole, not unfavourable in view of the fact that Hungarian literature

records five cases only where diagnosis has been made during life [16, 29, 33].

All eight cases reported reflect the diagnostic difficulty. In Case 2 it was the hyperacute clinical course, in Cases 1, 3, 6 and 8 the features imitating hepatic cirrhosis which masked the veno-occlusive process of the liver. In cases 3, 4 and 5 the polycythaemia was masked at first by the signs of portal hypertension, so that the correct interpretation of the signs suffered a certain delay. In cases 6, 7 and 8 the symptoms of the hepatic tumour were too closely linked with those of veno-occlusion; the latter condition could be differentiated in Case 7 only.

Treatment. Causal therapy could be considered in Cases 2, 4 and 5 in view of the results of ^{32}P treatment of the polycythaemia. As regards the benefits of this therapy from the aspect of the Budd—Chiari syndrome, it was found in fact to cause a significant remission in Case 5 still continuing to the present day.

We find three similar observations in the literature [10, 18, 29] in two of which [10, 18] the formation of a splenorenal or portacaval shunt, respectively, had to be performed, in addition to ^{32}P therapy.

In two of the present cases, ^{32}P failed to avert the occlusion of the hepatic veins. In Case 2, this complication ensued in a state of full haematological remission and in Case 4 it followed its fatal course in the presence of a favourable haematological response to ^{32}P.

As a *final conclusion*, the Budd—Chiari syndrome must be considered a rare condition which 125 years after BUDD's classical description, still often escapes detection. Intimate familiarity with the features of the syndrome is, in our view, still the best means for its diagnosis. Portal hypertension and pain in the hepatic region in the absence of a typical history should direct attention to its presence. In addition to the diagnostic difficulties, prognosis is nearly as poor as before. Improved diagnostic means for the early recognition of the obstruction of the hepatic veins and more efficient therapeutic weapons than those available at present might bring a change for the better in the outlook of the process.

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URINARY AND SERUM TRANSAMINASE LEVELS IN RATS WITH ORGANIC MERCURY POISONING

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An organic mercurial compound, methoxy-ethyl mercuric chloride (MEMC) has been studied for its toxic effect on the renal tubuli in rats. In view of the protracted nature and the slight degree of the effect, for its demonstration urinary transaminase activity was chosen. On the evidence of parallel determinations of serum GOT and GPT activity it could be ascertained that urinary transaminase activity is due to detached and disintegrated tubular epithelial cells. This was further supported by the presence in urine of large numbers of such cells. Neither the concentration and dilution tests, nor the protein and sugar content of the urine are sensitive enough for the detection of these slight tubular lesions.

Thus, MEMC, apart from its well-known toxic effect on the nervous system, has been found to induce renal lesions accessible to the function test employed.

Organic mercurial compounds form the basis of numerous pesticides and methoxy-ethyl mercuric chloride (MEMC in the following) has a nephrotoxic effect similar to that of corrosive sublimate [1].

It has been shown earlier that MEMC, even below the dose-levels involving somatic manifestations, interferes with previously formed conditioned nutritional reflexes in rats [2]. In our studies of acute MEMC poisoning BAL was less effective than against other organic mercurial compounds including sublimate [3]. The present study has been concerned with the nephrotoxic effect of MEMC. It is well known that neither the current renal function tests, nor the histologic procedures are sensitive enough for the detection of minor tubular lesions [4, 5, 6]. For this reason, ROSALKI and WILKINSON [7] suggested to estimate the urinary transaminase level for the demonstration of slight tubular lesions. In the present study it was sought to ascertain whether the said enzyme was a reliable indicator of the minor tubular lesions induced by MEMC.

Material and method

A total of 132 male albino rats weighing between 150 and 180 g was used. The animals were kept on synthetic rat food. MEMC (Berk Limited, London) was administered intraperitoneally as a 1% solution in distilled water.

The following groups were set up.

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Group I. 72 animals were given 2 mg/kg MEMC six times weekly for six weeks. (The dose administered corresponds to one fifth of the acute $LD_{50} = 9.4 \pm 0.9$ mg/kg, determined earlier.)

Group II. 60 animals were given 0.2 mg/kg MEMC six times weekly for 12 weeks.

In both groups the following investigations were performed before and once weekly during treatment.

a) Glutamic oxaloacetic transaminase and glutamic pyruvic transaminase were estimated by the method of REITMAN and FRANKEL [8] in urine collected for 16 hours;

b) epithelial cell counts in the unstained urinary sediment, using a Buerker-chamber; Every third day the following tests were performed.

c) Concentration test, by refractometric measurement of the specific gravity of urine collected for six hours from animals kept for this time in special containers;

d) dilution test by measurement of urinary specific gravity every hour after the administration at 14 o'clock of 1.5 ml tap water per 100 g body weight through a stomach tube:

e) estimation of the serum GOT and GPT levels every week in a different group of four animals for each of the two substances, blood samples of 5 ml being obtained by heart puncture;

f) regular checking of the urine for protein (sulphosalicylic acid) and sugar (Benedict's test), and recording of body weight.

At the end of the experiment, the animals were killed by decapitation and their parenchymatous organs were submitted to histological study.

Results

In preliminary tests of 24 rats, the serum and urinary transaminase level remained normal in all animals throughout the entire period of 10 weeks. Pyruvic acid concentration in urine ranged between 5 and 7 μg, invariably below 10 μg, and in serum between 14 and 17 μg, in accordance with the figures given by BALÁZS et al. [4].

Group I. Urine. In response to MEMC, GOT and GPT activity exhibited a rise up to 30 μg until the third week, and a subsequent decline; at the end of six weeks the levels were in the neighbourhood of the normal value. At this point, administration of MEMC was discontinued, the subsequent changes in the transaminase levels were of no significance. The cell count in the urinary sediment revealed an increase which, similarly to the transaminase level, attained its peak in the course of the third week (Fig. 1). At this time, numerous cell fragments appeared in the urine, greatly interfering with the accuracy of the cell counts.

Dilution and concentration capacity, specific gravity, and sugar content showed no typical change but proteinuria was common.

Blood. An elevation of serum GOT and GPT was noted the first week. The increased values persisted throughout the entire experimental period and did not return to normal even after the administration of MEMC had ceased (Fig. 2).

Histology. With the progression of poisoning, the detached tubular epithelium was replaced by newly formed flat basophilic cells, presumably as a sign of epithelial regeneration.

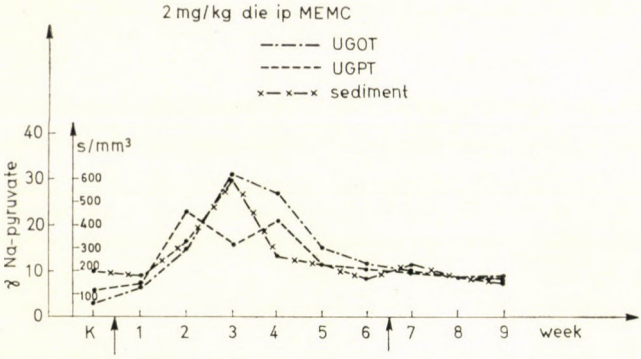

Fig. 1. Effect of 2 mg/kg/day of MEMC on urinary GOT and GPT level. Abscissa: *K* — control time of treatment in weeks. Ordinate: Na pyruvate (oxaloacetate), μg. Number of epithelia cells/ml in urinary sediment. Arrows: beginning and end of the treatment. In 2nd, 3rd and 4th week, $p < 0.02$; the deviation from the initial values was significant

Group II. Urine. Throughout the entire 12 weeks there was a continuous rise in the transaminase level from the initial values of less than 5 to 7 μg up to the 20 to 25 μg which were reached by the end of the 5th to 6th week to decline again from this time onward, returning to the original value by the end of the 12th week. There was a close parallelism between these changes and the increase in the cell counts in the urinary sediment, although the maximum of 300 cells per ml was attained in the third week (Fig. 3). The concentrating and diluting capacity as well as urinary sugar excretion remained unchanged; exceptionally, protein was found.

Blood. Serum GOT and GPT remained unaffected throughout the entire observation period of 12 weeks. The animals exhibited no pathological symptoms and gained in weight adequately.

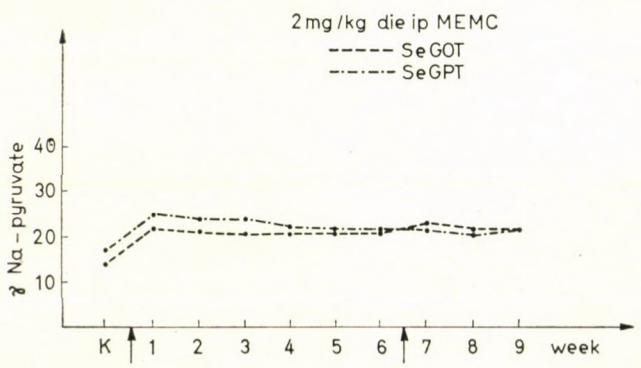

Fig. 2. Effect of 2 mg/kg/day of MEMC on serum GOT and GPT level. Abscissa: *K* — control; time of treatment in weeks. Ordinate: Na pyruvate (oxaloacetate), μg. Arrows: beginning and end of the treatment. Throughout the entire period, $p < 0.02$

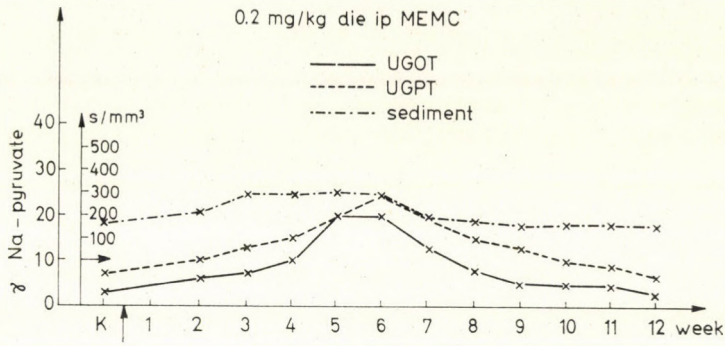

Fig. 3. Effect of 0.2 mg/kg/day of MEMC on urinary GOT and GPT level. Abscissa: *K* — control; time of treatment in weeks. Ordinate: Na pyruvate (oxaloacetate), μg. Arrows: beginning and end of the treatment. Between the fourth and eighth week, $p < 0.02$; the deviation from the initial values was significant

Discussion

The urinary transaminase level seemed to be a suitable index of the slight tubular injury induced by chronic mercury poisoning. The procedure requires a small amount of urine, thus permitting to perform repeated tests, an advantage in toxicological practice, since it offers a convenient means of checking the progress of slight tubular lesions and provides a specific test for the demonstration of early tubular damage.

MEMC has been found to induce tubular lesions. Application of minor doses results in detachment and elimination with urine of the tubular epithelial cells, corresponding to the extent of the injury. The disintegrating cells release transaminase, thus increasing its urinary level. Since both GOT and GPT have been found to maintain their original concentration in the serum, the increased urinary transaminase level can have no other source than the kidney.

After administration of major doses of MEMC, the serum transaminase level also increases. This may be accounted for by two possibilities; either the liver is affected by MEMC, myocardial damage caused by the substance having been practically excluded [9] or there is a return of urinary transaminase into the serum.

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BRONCHIAL OAT-CELL CANCER PRODUCING ACTH, SEROTONIN AND CATECHOLAMINE

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A case of bronchial carcinoma producing ACTH, serotonin and catecholamine is reported. There has been thus far no evidence of the production of hormones of non-polypeptide character in bronchial carcinoma. The clinical signs and symptoms were consistent with catecholamine-overproduction.

There has been increasing evidence in the literature on hormone-producing ectopic tumours [27]. Between 1966 and 1970, the reported cases of ACTH and ADH-secreting tumours as well as of those associated with hypercalcaemia increased from approximately 150, 27 and 37, respectively, to 175, 40 and 52. This increase which is far beyond the likewise progressing incidence of malignancies is also valid for tumours secreting hormones of other kind, e. g. gonadotropin, GH or serotonin. This might be due to a growing awareness of the fact that the cases of this kind, regarded earlier as incidental, have a connecting link, the production of hormones by non-endocrine tissue. Meanwhile the number of reported cases of ectopic tumours with multiple hormone secretion has also been on the increase. The following combinations are known: ACTH + MSH [7], ACTH + ADH [30], ACTH + aldosterone [14], ACTH + MSH + gastrin [11], ACTH + parathormone-gastrin [13], catecholamine + erythropoietin [24], TCT + ACTH [4], insulin (ILA) + erythropoietin [23], insulin (ILA?) + serotonin [28], insulin (ILA?) + erythropoietin + parathormone [1], HCG + TSH [9], finally, serotonin + gastrin [17]. All of these hormones produced in ectopic non-endocrine tissues are polypeptides, with the exception of serotonin and 5-hydroxy-tryptophan [6] which, however, may result from the breakdown of tryptophan and represent thus metabolites rather than products of synthesis.

In the only reported case of an allegedly aldosterone-secreting oat-cell carcinoma [14], apart from clinical, i.e. indirect, signs, no direct proof has been adduced of the tumour's aldosterone production. But even if an increase in the aldosterone level had been ascertained, it need not have been due to the tumour itself, since the increased ACTH level present in this case resulted in a hypertrophy of the adrenal cortex.

Nor did the reported case of pheochromocytoma associated with polycythaemia [24] necessarily imply that the tumour has been producing a polypeptide (erythropoietin) in addition to the catecholamines though theoretically this is certainly possible. It seems, however, more reasonable to seek the primary cause of the enhanced haemopoiesis in the anoxaemic effect of the catecholamines on the kidney.

With a few exceptions including the Zollinger—Ellison-syndrome, ectopic hormone production is a property of malignant tumours.

One of the interpretations accounting for ectopic hormone secretion regards the polypeptides of hormonal activity produced as "by-products" of an enhanced protein uptake and breakdown by the malignant cell. This is in line with the fact that most of the substances thus produced are immunologically different from the physiological hormones, and it is only in their biological effects that they are identical.

The other theory [22] connects the phenomenon under study with a release from genetic repression (de-repression) as a result of a mutation affecting the genetic information of tumour cells. The possibility of a de-repression taking place in the primary cell constituents of the tumours is consistent with the fact that it is invariably the same polypeptides which are formed by a given tumour, and this also applies to the metastases formed after the elimination of primary tumour. This theory also implicates that for the synthesis of hormones with a cyclic (steroid) structure the respective endocrine organ must be in possession of certain specific enzymes. However, we find it hard to reconcile the mutation-de-repression theory with the observation that the unusual hormone synthesis is almost exclusively linked to a particular histological structure. For instance bronchial oat-cell carcinoma produces ACTH and ADH, squamous cell carcinoma TSH, macrocellular adenocarcinoma gonadotrophin, non-beta cell pancreatic adenoma gastrin, cerebellar haemangioma secretes erythropoietin, a haemangioma of other sites a humoral factor of PTH-nature [26]. These relationships are too close to be due to chance and suggest instead that mutation is limited to a certain extent by the cell type.

In the case to be reported, the hormone detected in the ectopic tumour was, contrary to all other observations, catecholamine, and the clinical features were typical of catecholaminaemia. Thus, the case may represent a hitherto unpublished variety of ectopic hormone production.

Case report

M. J., a 49-year-old male was admitted on February 5th, 1968. His family history was irrelevant. He was a heavy cigarette smoker. For the last three months he had had intensive pains in the chest and coughing spells, swelling of the ankles in the evenings and nycturia. Occasionally, he had experienced crises of palpitation accompanied by tremor and perspiration. Seen by his physician on one of these occasions he was found to have a high blood pres-

sure. One month later he had felt a sudden sharp pain in the region of the left costal arch, attributed to an infarct of the spleen. He had a marked dyspnea lately and had been losing weight. During an earlier admission, X-rays had revealed a fist-sized infiltration in the region of the left hilus suggesting a pulmonary tumour or an aortic aneurysm.

On admission there was a marked generalized pigmentation of the skin, slight ankle oedema, hoarseness and stridulous breathing. Percussion revealed a palm-sized dullness over the left lung and restricted diaphragmal motion. The left hypochondrium was protuberant and tender to percussion. The lower pole of the spleen was palpable. The patient was emaciated, weak, he exhibited repeated runs of ectopic beats. Blood pressure was 115/50 m Hg; ESR, 20 mm/hour; RBC, 2 500 000 (?); WBC, 7800; serum Na, 146; serum K, 2.5; serum Cl, 107 Meq/l; blood sugar, 104 mg; endogenous creatinine clearance, 93 ml. ECG: auricular and ventricular ectopic beats, diphasic T and depressed ST in standard leads I—II; urinary catecholamine excretion, 60 μg/24h. Chest X-rays revealed a fist-sized intensive intrapulmonary mass with sharp outlines, attaching to the upper pole of the left hilus and sending out several extensions into the surrounding area. On the sagittal tomogram it was found to cause narrowing and downward displacement of the segmental bronchus (Fig. 1). The bronchus of segment 3 could not be visualized (Dr. Bartha). Laryngoscopy revealed thickening and almost complete immobility of the left vocal cord with hyperaemia in its surroundings. Bronchoscopy: slight displacement to the left of the left main bronchus as also of that of the upper lobe with free orifices and minor inflammatory signs of the mucosa. Owing to the bronchial displacement to the left, inspection of the bronchial lumen on this side was not possible.

Clinical course. On February 9, the patient experienced repeated hypertensive crises up to 190/110 mmHg. Phentolamine produced a fall to 140/70 within 30 seconds, the effect lasted however 4 or 5 minutes only, then the blood pressure rose again. On February 10, he went into a comatous state with hyperpyrexia, sluggish reflexes, followed by pulmonary oedema, Cheyne-Stokes respiration and death.

Post mortem the heart weighed 440 g, the left ventricular wall was 18 mm thick. The hilus of the left lung was occupied by a lobulated tumour of 170 g weight, partly protruding from the pulmonary parenchyma toward the mediastinum, partly extending into the pulmonary tissue and enclosing the left main bronchus to the degree of narrowing its lumen. On its cut surface the tumour appeared soft, friable, and in places liquified; it was greyish-white in

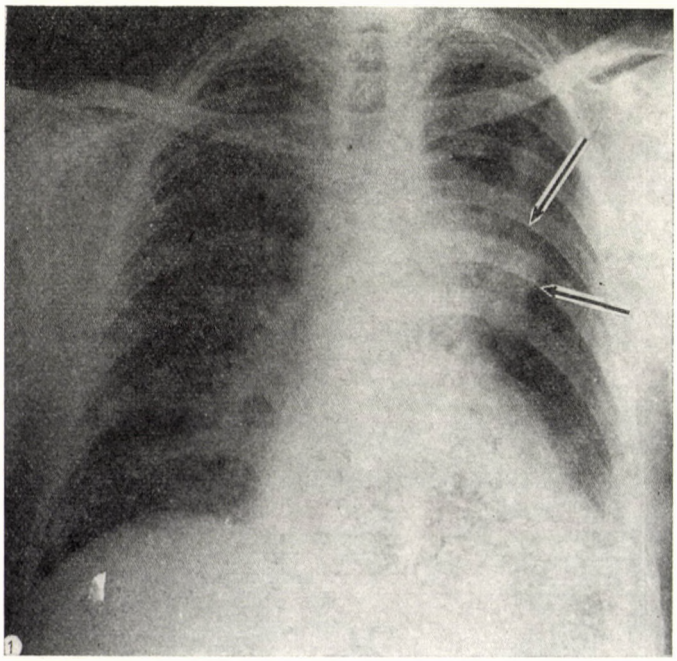


Fig. 1

colour with purplish-red patches (Fig. 2a). There was pulmonary oedema. The spleen weighed 250 g, it was bright red in colour and firm in consistency. The two adrenals weighed 50 g; on the cut surface the cortical area measured 2 to 3 mm in breadth, the medullary substance was liquified (Fig. 2b). The cortex was densely packed with bright whitish-yellow miliary nodules. The thyroid, pituitary, pancreas and pelvic organs revealed no gross abnormality. Histological study: the cells of the pulmonary tumour are small, poorly differentiated, being typical of "oat cell carcinoma" (Fig. 3). The nuclei form partly round, partly tapering hyperchromic irregular aggregates or groups. The connective tissue septa are poor in cells; there are scattered necrotic and haemorrhagic foci. The original structure of the adrenals is recognizable but the cortical area is broadened. The zona glomerulosa is dissociated, the zona fasciculata has a variegated appearance: the gross whitish-yellow areas correspond to small poorly demarcated adenomas formed by large, light, lipid-containing cells of foamy structure. There are also sporadic groups formed by cells with eosinophilic, finely granulated cytoplasm (Fig. 4). The cortical cells in the neighbourhood of the medulla contain finely dispersed brown pigment

Fig. 2a

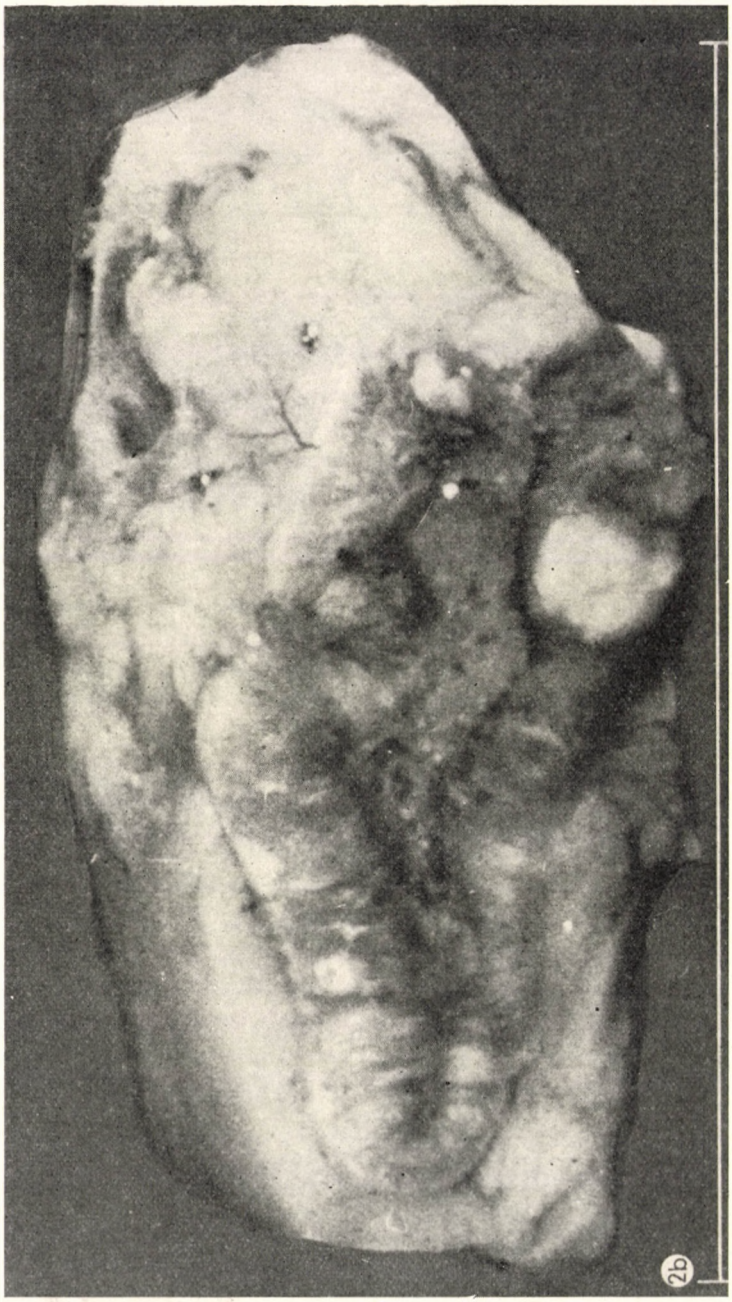
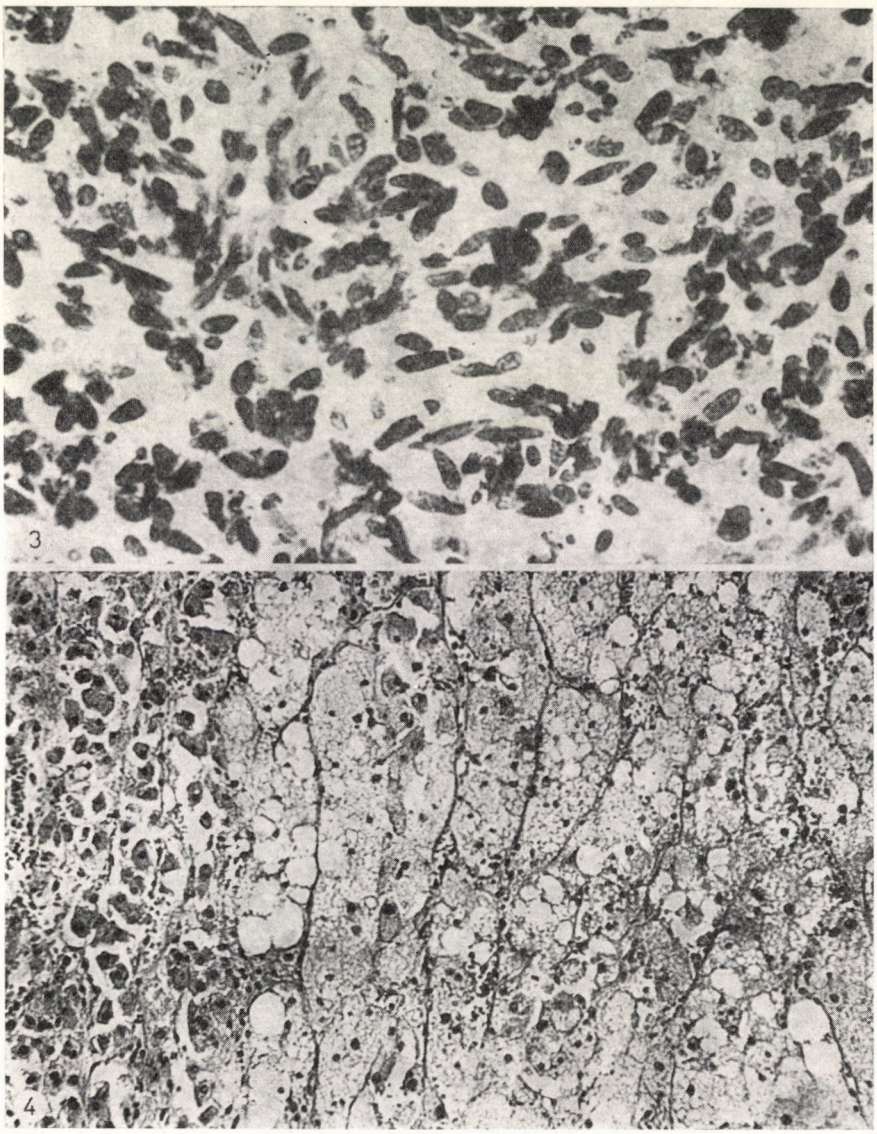


Fig. 2b



Figs 3 and *4*

granules. The medullary cells are polymorphic. The anterior pituitary lobe exhibits collections of secretion of concentric structure with acidophilic staining character (M. Balázs).

In addition to the microscopic features of the tumour, its hormone-producing characters have been studied.

Methods

The tumour was extracted according to LYONS [15] with 80% acetone + 0.2 M HCl for 2hrs. Subsequently, 10 parts of acetone were added to the supernatant and the precipitate was redissolved in water and lyophilized. The resulting substance weighed 102 mg. Its biological effectiveness was determined by Opton spectrofluorometric rat corticosterone secretion test as described by PURVES and SIRETT [23a]. The biochemical identification of the active substance was assessed by gel electrophoresis.

The catecholamine content of the tumour was estimated by the method of HINGERTY [9b], its serotonin content by that of HANSON and SERIN [7a].

Results

I v per 100 g rat weight	ACTH (3rd Int. Working Standard) mU			Tumour extract μg		
	1	1,5	4,5	1,7	5	15
Corticosterone μg/100 ml plasma	21	36,5	42	19	34	40,5

Calculated from the above data the tumour extract contained 245 mU ACTH activity/mg weight (Gy. Hajós).

Gel electrophoresis revealed 8 to 10 peptide components, one of them in the zone of natural porcine and synthetic human ACTH [2]. The biological activity of the primary material and its electrophoretic localisation in the basic areas made its similarity, if not its identity, with ACTH highly probable.

The tumour contained 75 μg/g dry weight of catecholamine, a total amount of 12,750 μg. Under physiological conditions the concentration of adrenaline and noradrenaline together is 260 to 1000 μg/g, thus, taking the average adrenal weight and the usual cortico-medullary proportion (90 : 10) into consideration, the total content of the entire organ may be estimated at 470 to 1800 μg. The total amount in the present case, in the non-enlarged medulla was far in excess of this value, i.e. 6000 to 7000 μg, suggesting an inhibition of medullary release. The total amount of serotonin was 98 mg, or 576 μg/g.

Discussion

It is generally known that the clinical manifestations of hormone-producing tumours originating from non-endocrine tissues are often different from those resulting from an excessive but none the less physiological output of the same hormone. For instance, ACTH-producing bronchial carcinoma, though being generally associated with excessive cortisol levels, hypopotassaemia, muscular asthenia, reduced glucose tolerance, oedema, hypertension as well as with a reduction in the ACTH-reserves of the anterior pituitary lobe as a result of the feedback mechanism by the elevated plasma cortisol levels and by the ectopic ACTH-production [16]. On the other hand, obesity, striae, osteoporosis are lacking, probably because the time is generally too short for their production [10] (here may, however, be an abnormal pigmentation unusual in Cushing's disease). In the present case, qualitative and quantitative

studies of ectopic hormone-production were limited by the shortness of the observation period and by the terminal state of the patient, therefore, in addition to hyperpigmentation it was only the presence of hypopotassaemia in the absence of hypochloraemia which indicated a definite increase in ACTH production. On the other hand, the hypertensive crises were suggestive of some additional hormonal activity, and distinctly pointed to a secretion of catecholamine or serotonin. As to the first alternative, the result of the phentolamine test was inconclusive. The fall in blood pressure, usually of at least 30 min. duration ceased within 15 min. although the decrease by 25 to 30 mmHg met the criteria of positivity.

The pathological and histochemical findings provided additional proof of the ectopic hormone secretion. A broadening of the adrenal cortex to more than 2 mm is in itself indicative of the gland's hyperplasia; their homogeneous enlargement to 50 g, greatly exceeding the 18 g considered normal, was still more conclusive [13]. The polymorphism of the cells of the zona fasciculata as well as the adenomas may be connected with ACTH stimulation and the eosinophilic, PAS-positive intercellular accumulations in the anterior pituitary lobe may also have a causal significance, since these would seem to represent deposits of glycoprotein material originating from other sites. In cases of ACTH producing ectopic tumours, hyaline degeneration of the basophilic cells (Crooke-cells) is a common observation; in the present case, however, neither such cells, nor hyperplasia of (or formation of adenoma by) the pituitary cells was demonstrable. In another case of bronchial carcinoma associated with Cushing's syndrome [8], Crooke-cells were likewise absent from the hypophysis.

A chemical study of the tumour-homogenisate for ACTH provided direct proof of ectopic hormone production. In the cases known from the literature the organ contained 0.01 to 5950 mU ACTH/g [13], and in the present case, 250 mU ACTH/g.

There was no basis for assuming that the symptoms would have been due to an adrenocortical tumour of mixed (cortico-medullary) type or that some hyperplasic medullary cells would have penetrated the cortex [12].

The earlier view attributing to certain tumours the capacity of absorbing externally produced hormones in a sponge-like fashion, which would account for the high hormone concentration in their tissue, is no longer tenable [27]. But even if absorption could take place, this would certainly not be true in the present case since it is hardly possible that a patient with normal catecholamine excretion and normal clearance should be capable of accumulating in a single tumour more than twice the amount of hormone contained in the adrenal medulla, the less so as the half-life of catecholamines, even in a "stable" binding, is as short as one day [21].

The size of the tumour may in certain respects determine the clinical manifestations. In tumours weighing less than 5 g the rate of synthesis is

very fast, binding is slight, so that the release is intensive. Consequently, the crises are continuous or follow in close succession, and VMA excretion is slight. On the other hand, in large tumours, binding of the hormone to proteins is strong, its release is slow, breakdown is enhanced and the VMA/Catecholamine ratio in the urine is high. In the present case there was no time for the determination of this value and these facts have been derived from observations in pheochromocytomas of chromaffin cell origine [31]. Since, however, we have to deal with facts which follow from the physico-chemical conditions of the tumour, they must likewise be true for tumours of ectopic hormone production.

We might furthermore consider the possibility that the corticosteroids potentiate the activity of phenyl-ethanol-N-methyltransferase, an enzyme involved in noradrenalin adrenalin transformation [18], although this would merely affect the proportion between the catecholamines rather than their total amount.

The possible role of an inverse potentiation by a medullary tumour inducing cortical hyperplasia has also been taken under consideration, but not in the present case where the medullary origin of the tumour was definitely ruled out by the histological findings. In the light of the laboratory results, it seems far more probable that the catecholamines detected in the tumour have been produced locally. In the accessible literature there is but a single case similar to ours in this respect [27], with the difference, however, that the tumour was a bronchial carcinoid with argentaffin, chromaffin and argyrophil elements and it contained 0.5 μg per g tissue of catecholamine with a total of 60 μg. In the present case, neither these histologic features nor a neuroblastic proliferation of any kind were demonstrable.

The total amount of serotonin found in the present case also exceeded the 5-HT content described as normal [3, 6, 18, 29] and it was only in the case reported by SMITH [26] where we found higher values, i.e. 4,000 μg/g. The question may arise whether the high serotonin concentration might be connected with the secretion of ACTH, the more so as cortisol enhances the activity of tryptophan pyrrolase, a hepatic enzyme involved in the breakdown of tryptophan [5]. An inverse relationship may also be considered *viz.* in the course of tryptophan and tyrosine metabolism derivatives of tryptamine and of catecholamine may well be formed simultaneously within the same system as a result of enzymatic breakdown. Although this possibility cannot be ruled out altogether, the excessive amounts found in the tumour make it more likely that there the synthesis of the hormones takes place independently of each other. If this is the case (the benign bronchial carcinoid referred to earlier [27] being disregarded), it was for the first time that beside ACTH and serotonin a hormone of non-polypeptide structure has been found in an oat-cell bronchial carcinoma.

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INHIBITION OF SKIN ALLOGRAFT REJECTION BY ANTILYMPHOCYTIC SERUM PRETREATMENT OF THE DONOR

IMMUNOLOGICAL PREPARATION OF THE GRAFT

By

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Pretreatment of the donor with antilymphocytic (antithymocytic) serum has been found to prolong the survival of mouse-skin allografts. The effect is attributed, in addition to a suppression of the immune activity of the lymphocytes in the donor tissue, to the presence of graft-protective antibodies.

The fact that the individual's histocompatibility antigens being represented in his leukocytes, lends itself to studies in mixed lymphocyte cultures [2]. In the case of incompatibility in vivo, the intradermal injection into the donor of the recipient's slymphocytes results in an inflammatory reaction at the site of normal lymphocyte transfer (NLT-test) [4]. It is furthermore known that the lymphocytes are continuously circulating between the blood and the tissues, therefore a part of the lymphocyte population is always in the tissues.

Presuming that in allografts an inflammatory reaction of a mechanism similar as in the NLT may arise in the vicinity of the donor lymphocytes and thus contribute to the rejection of the graft, we have examined in the present study whether pretratment of the donor with antilymphocytic serum (ALS) was capable of inhibiting the rejection of the graft.

Material and method

For ALS an antithymocytic serum (ATS) was used which had been produced in two goats (I and II) against thymus-cell suspensions of 20 day old AKR-mice and exhausted for anti-mouse-erythrocyte antibodies. ATS of goat I revealed a considerably higher activity than that of goat II (Table I), therefore this serum was actually used in the studies. Transplantation was performed in female mice, auricular skin of donor Balb/c mice being transferred into the back of recipient CBA mice. The allograft measured approximately 8×10 mm. Two control groups were set up, one untreated, the other treated with normal goat serum in the same manner. For the demonstration of the effect of ATS, the serum was given to one recipient group only. Pretreatment of the donors consisted in the intraperitoneal administration of 0.1 ml ATS, three times, i.e. 72, 48 and 24 hrs before the transplantation. In the first part of the study we examined the survival of the graft of pretreated donors, and in the second, ATS wasgiven to the recipients too, in the same manner as to the donors with the difference that they received an additional dose of 0.05 ml ATS 24 hrs after transplantation.

Table I

Comparative data relative to goat anti-mouse ATS I and II
In the absence of ATS-treatment, survival of skin grafts was 11.7 ± 1.4 days (see Table II)

	Lymphocyte agglutination titre	Lymphocyto-toxicity titre	Survival of skin grafts in ATS-treated recipients (in the H_2 system)
I ATS	1 : 2048	1 : 256	18.5 ± 1.4 (SD)
II ATS	1 : 1024	1 : 128	15 ± 1.2

Results

Mean survival of the graft was

1. in the untreated mice, 11.7 ± 1.4 days;
2. in the recipients treated with normal goat serum, 12 ± 1.2 days;
3. with ATS I preatreated donors, 14 ± 1.5 days;
4. in the recipients after treatment with ATS II, 15 ± 1.2 days; and after treatment with ATS I, 18.5 ± 1.4 days;
5. after pretreatment of the donors and treatment of the recipients with ATS I, 22 days. In view of the fact that inbred syngeneic animals of identical sex and similar body weight were used, the difference revealed by the one-sample *t*-test between groups 1 and 2 was not significant ($p < 0.5$), while it was significant between groups 1 and 3 (p 0.001) and strongly so between groups 1 and 5 (p 0.0001). The suppressive effect of the recipients' ATS-treatment on graft rejection was significantly enhanced by ATS-pretreatment of the donor.

Thus, ATS-pretreatment of the donor mice significantly prolonged the retention of the skin graft by the recipient, furthermore, a combined treatment of the donor and the recipient resulted in the longest graft survivals.

Discussion

It has been assumed that passenger donor lymphocytes present in the tissues are inevitably transferred together with the graft, and thus endanger its survival by giving rise to an inflammatory reaction similar to that associated with the normal lymphocyte transfer test (NLT). The local antilymphocytic reaction is also of two types: it may be a local graft-versus-host (GvH) and a host-versus-graft (HvG) reaction. The NLT-reaction is suppressible by pretreatment with ALS of both the donor and the recipient. Irradiation of the donor or of the donor tissue may also prolong the survival of the graft to some extent. It is furthermore known that pretreatment of the donor with ALS

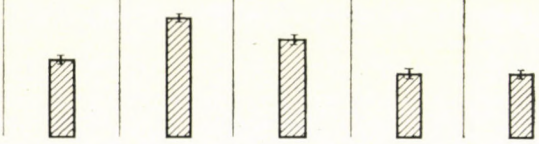
or incubation of bone-marrow grafts in ALS reduces the intensity of GvH reaction, and of the "secondary disease" (runt disease) [1, 8].

The present results thus seem to support our claim that ATS-pretreatment of the donor is inhibitory to the immunocompetent, lymphoid cells present in the donor's tissues and has therefore a graft-protective effect. However, the only fact positively ascertained on the evidence of our findings is the graft-protective influence of ATS-pretreatment, but whether this effect was actually due to a suppression of the passenger donor-lymphocytes occurring in the graft, has yet to be controlled. In earlier studies we have found that after absorption with erythrocytes ATS still possesses antibodies against various other tissue antigens aside from the circulating lymphoid cells or

Table II

Survival of skin grafts $(BALB)_c$ CBA) after treatment with ATS I

	ATS-treatment of donors	ATS-treatment of donors and recipients	ATS-treatment of recipients	Treatment of recipients with normal goat serum	No treatment
Graft survival (days)	14 ± 1.5 (n = 10)	22 ± 1.3 (SD) (n = 11)	18.5 ± 1.4 (n = 10)	1.2 ± 1.2 (n = 9)	11.7 ± 1.4 (n = 45)



thymocytes [5] and that it reacts with antigens which are not confined to these lymphoid elements. It has been shown in renal allotransplantation studies in rats [7] that from the antithymocytic gamma globulin used for the pretreatment of the donor strong antibodies become attached to the kidney and are thus transferred to the recipient together with the organ; however, non-purified ATS may cause a typical complex immune nephritis. On the other hand, the opposite of this, i.e. specific immunosuppression with practically unlimited survival of the graft has been achieved by other types of immune transfer [10]. It may be assumed even on the evidence of rat studies that the antibodies confer a protective effect on the skin [3]. Therefore it is not, or not only, the specific antilymphocytic or antithymocitic activity of ATS which accounts for its effect on graft retention but the activity of graft-protective, enhancing antibodies fixed to the donor tissue [11] also have their

part. It has long been our belief that the effect of some autoantibodies is protective rather than aggressive [9] and we intend to separate this mechanism into its components by further studies. This duality may well account for the difficulties of ALS or ATS standardization as also for the observation that non-purified ATS may be occasionally of greater activity than highly purified preparations having been absorbed with other antigens.

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PAROTID RESPONSES TO INDIRECT STIMULATION IN PATIENTS WITH DEPRESSIVE ILLNESS

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Parotid secretion has been investigated in 20 depressed patients (6 males and 14 females, mean age 41 years, one belonging to the reactive, 19 to the endogenous type of depression), against 15 non-depressed controls (10 males and 5 females, mean age 33 years) prior to therapy and after recovery. Saliva was collected from the parotid by means of a plastic capsule, the intensity of secretion being measured by the rate of drops per minute. In the depressed patients, parotid secretion whether under basal conditions or in response to nearly all stimuli employed (unilateral mechanical and chemical stimuli, "simultaneous summation", "successive summation", serial stimuli by means of 5 ml 5% citric acid) suffered a significant reduction. Dehydration as a potential antisecretory factor was ruled out. The increase in secretion was inversely related to the intensity of the stimulus. The initially low secretion increased parallel with clinical improvement without, however, attaining the control value even after recovery.

Most investigations into the pathogenesis of depression have been focussed on the significance of catecholamines. The possible involvement of cholinergic mechanisms and of their disorders has received little attention, though it has been pointed out by STRONGIN and HINSIE [16] that parotid secretion of predominantly parasympathetic innervation is significantly reduced in depressed patients as compared with those with manic disease or with healthy controls.

Though investigation of the parotid function is not expected to yield results of universal validity, in view of the relative simplicity of the procedure (the parotid gland being an organ of particular responsiveness), it none the less seemed to lend itself to studies of the cholinergic mechanisms [1].

The data published in the literature are inconsistent and even contradictory. While PECK (1959) found definite correlations between the severity of depression and the reduction in parotid secretion, BUSFIELD and WECHSLER (1961) deny any relationship between the intensity of parotid secretion and the grade of depression. Again, DAVIES and PALMAI (1964) reported that basal parotid secretion significantly diminished with the severity of depression and rose parallel with clinical improvement, its values after clinical cure being identical with those of normal subjects. In contrast, a reduced parotid secretion

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was noted by GOTTLIEB and PAULSON (1961) to accompany the onset of the process and to persist even after recovery, regardless of sex, type of depression, and its therapy.

According to the findings of LOEW (1965) it is in circular psychosis where basal parotid secretion is the lowest, lower than either in the psychogenic or in the involutionary type of depression. Anxiety and agitation reduce basal parotid secretion to a greater extent than do inhibition and apathy.

In the depressed subjects not only basal parotid secretion but also its response to citric acid stimulation was significantly reduced, as compared with the controls (DAVIES and GURLAND, 1961).

The pertaining data concern for the greatest part the basal secretion or (DAVIES et al. 1961, 1964) the responses to citric acid or to Methacholine stimulation.

In the present study indirect stimulation of different types and variable intensity was performed in order to obtain information on certain points, in particular whether the intensity of secretion undergoes any change as a result of clinical improvement.

Material and methods

The study included 20 depressed subjects (14 females and 6 males from 18 to 77 years, mean 41 years of age). One of the patients belonged to the reactive, and 19, to the endogenous type of depression. In one case depression was of minor, in 6 cases of medium, and in 15 cases of major severity. With the exception of one case, all had been under our care as inpatients several times before, the diagnosis having been depressive psychosis in all instances. Agitation occurred in none, anxiety in one of the cases. Drug therapy and ES were withheld for the period of study. Six and ten patients were repeatedly tested after full clinical cure.

The control group was made up of 15 nondepressed subjects, 10 males and 5 females between 21 and 60 (mean, 33) years of age, all having been admitted for lumbar spondylosis or a slipped disk.

For the collection of saliva a plastic capsule provided with an outlet was placed over the opening of the parotid duct; for the purpose, a vacuum-fixed Lashley capsule (LASHLEY 1916, SZABÓ 1965, MÓZSIK 1966) has been adapted. In opposition to the original the capsule had a single compartment and was held in position by a mechanical device (LIPÁK 1968) (Fig. 1).

The intensity of secretion as reflected by the number of drops per minute was registered by an EEG-apparatus and an electric drop counter. In every subject the following parameters were registered:

a) basal secretion prior to stimulation,

b) response to mechanical stimulation applied first to the right, subsequently to the left anterior two thirds of the tongue for 20 seconds (trigeminal-glossopharyngeal reflex);

c) responses to chemical stimulation by the application of a gauze-pad soaked in 5% citric acid to the same areas of the tongue and in the same order as under *b)* for 20 seconds (facial-glossopharyngeal reflex);

d) response to a single citric acid-stimulation by placing an 5% citric acid solution into the patient's oral cavity and leaving it to be swallowed after 20 seconds. Since this procedure provided a synchronous stimulation of the trigeminal, facial, glossopharyngeal and vagal receptors, its effect was evaluated as that of a "simultaneous summation";

e) the former stimulation was repeated one minute later ("successive summation");

f) serial stimulations including three citric acid tests at two-minute intervals.

The order of succession, times and place of the tests were invariably identical.

Mathematical analysis of the results was based on the arithmetical means and standard deviation, significance was evaluated by the one- and two-sample t test and, where this was possible, by the F-test, otherwise by the d-test.

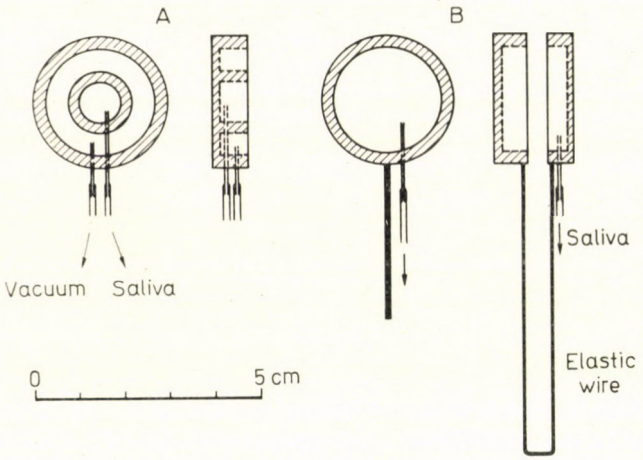

Fig. 1. Capsules for the collection of parotid saliva. *A.* The conventional Lashley-capsule with vacuum fixation; *B.* Capsule with mechanical fixation used in the present study

Results

a) Basal secretion. The average drop rate per minute (1.8) was lower in the depressed subjects than in the controls (11.0), and showed a significant ($p < 0.001$) rise after cure (5.4) as compared with the pre-treatment values (2.7), remaining, however, lower (5.4) than in the non-depressed controls (11.0) (Fig. 2, Table I).

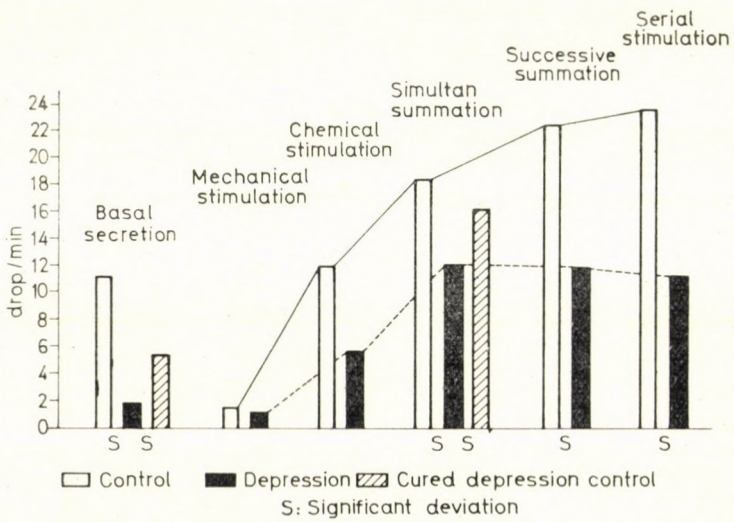

Fig. 2. Parotid secretion under basal condition and its response to stimuli of various types (average number of drops per minute) in depressed and in control subjects prior to treatment and after recovery

Table I

Mean resting parotid secretion and standard deviation (drops per minute) in the controls ($N = 15$) and in the depressed subjects ($N = 20$); resting secretion of depressed subjects before treatment and after recovery

Group	N	Resting secretion Drop/minute		Difference between the two groups		
		Mean	SD	no. of drops	per cent	p
Control	15	11.0	3.6	−9.2	83	$0.05 > p > 0.02$
Depressed	20	1.8	1.6			
Depressed						
before treatment	6	2.7	0.8	+2.7	100	$0.001 > p$
after recovery	6	5.4	0.4			

b) The "real" response to mechanical stimulation, i.e. the number of drops per minute produced in addition to the basal secretion rate, revealed no appreciable difference between the depressed patients (2.5) and the controls (1.4) (Fig. 2, Table II). No significant difference was found between the ipsilateral and contralateral responses, only the peak of the contralateral response occurred later in time (Fig. 3).

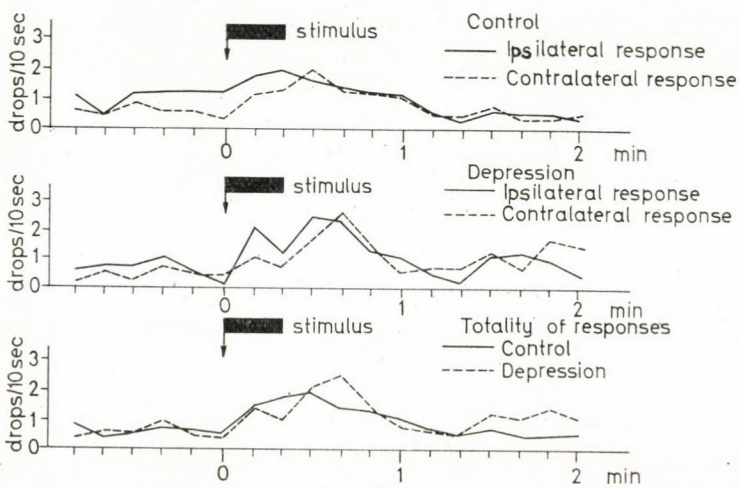

Fig. 3. Ipsi- and contralateral parotid responses to hemilateral mechanical stimuli applied to the tongue, plotted against time in controls (upper part) and in depressed subjects (middle). Course of all responses in time in the two groups examined (lower part)

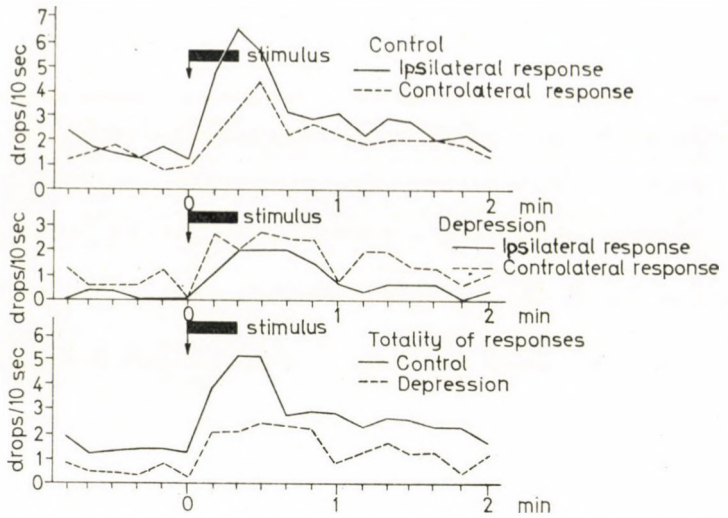

Fig. 4. Ipsi- and contralateral parotid response to hemilateral chemical stimuli applied to the tongue, plotted against time in controls (upper part) and in depressed subjects (middle). Course of all responses in time (lower part)

c) The response to hemilateral chemical stimuli displayed a distinct difference between the depressed subjects (5.5 drops per min) and the controls (11.7 drops per min), it being in the neighbourhood of the limit of statistical significance ($0.10 > p > 0.05$) (Fig. 2, Table III). The drop-rates of the contralateral responses were reduced and their peaks ensued later in time than those of the ipsilateral responses (Fig. 4).

d) Simultaneous summation. In the case of a single citric-acid-stimulation the "real" response of the depressed subjects (37 drops per 3 min) was significantly reduced ($0.05 > p > 0.02$) as compared with that of the controls (53 drops per 3 min) (Fig. 2, Table IV). The responses of the patients after the cure of depression were significantly ($0.01 > p > 0.001$) increased as compared with the pre-treatment values (47 drops per 3 min as against 34 drops per 3 min) without, however, attaining the control value (58 drops per 3 min). The responses are presented against time in Fig. 5.

e) Successive summation. The response of the depressed subjects to two citric-acid stimulations at a one-minute interval was still more significantly reduced ($0.05 > p > 0.02$) with reference to the controls than in the former group (47.3 drops per 4 min as against 89.5 drops per 4 min) (Fig. 2, Table V). The responses against time are presented in Fig. 6.

f) Serial stimulation yielded the most significant differences ($p < 0.001$) in the response between the depressed and the control subjects (89.9 drops per 8 min as against 185.3 drops per 8 min) (Fig. 2, Table VI). The responses are presented in Fig. 7.

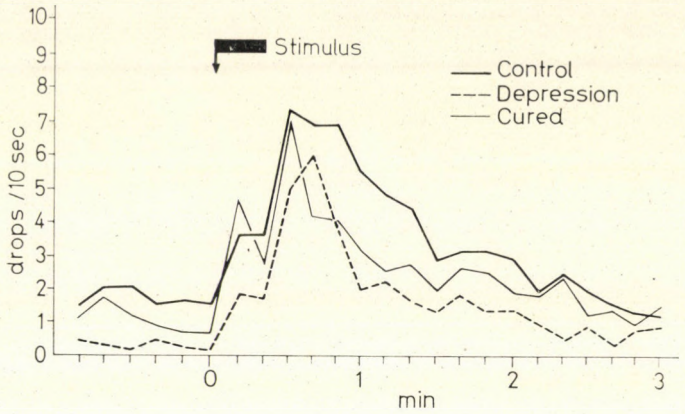

Fig. 5. Parotid response to stimulation with 5 ml 5% citric acid for 20 sec ("simultaneous summation"), plotted against time in controls and depressed subjects, before and after treatment

Discussion

From the findings the following conclusions have been drawn.

1. In agreement with published evidence, the parotid secretion of depressed subjects revealed a significant reduction under resting conditions as well as in response to various types of indirect stimulation, mechanical stimulation being the only exception.

In the reduction of secretion, several factors may have had a role.

a) Though certain factors pointed out by PECK (1959) and LOURIE (1943) to modify parotid secretion (e.g. drugs, time of meals, temperatures,

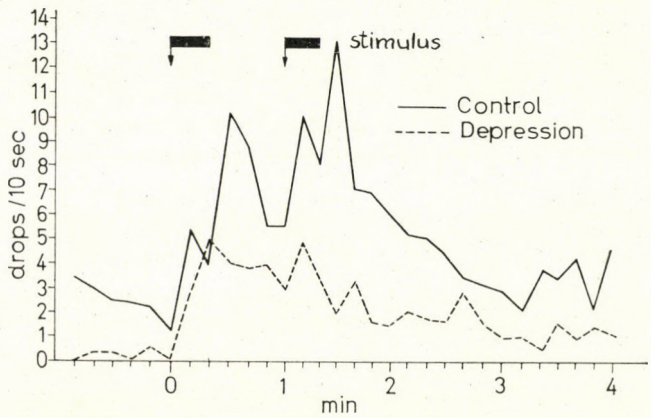

Fig. 6. Parotid response to two successive citric acid stimuli at an interval of one minute ("successive summation"), plotted against time, in depressed and non-depressed subjects

Table II

Ipsi- and contralateral parotid secretory response to hemilateral mechanical stimulation of the anterior two thirds of the tongue for 20 sec (mean number of drops per min), in the controls (N = 10) and in depressed subjects (N = 10)

The "real" response is the deviation from the pre-stimulation values expressed in drops per min.

Group	N	Drop/min			„Real response" per minute during stimulation				
		before	during stimulation	after	Drop/min	SD	Deviation between the two groups		
							no. of drops	per cent	p
Ipsilateral response									
Control	10	8.2	10.5	6.9	2.3	5.7	−1.1	47	$0.80 > p > 0.70$
Depressed	10	5.7	6.9	4.4	1.2	8.6			
Contralateral response									
Control	10	8.5	8.6	6.6	0.1	6.5	+3.9	3900	$0.20 > p > 0.10$
Depressed	10	3.4	7.4	4.8	4.0	5.7			
All responses									
Control	10	7.7	9.1	6.7	1.4	5.4	+1.1	78	$0.70 > p > 0.60$
Depressed	10	4.6	7.1	4.6	2.5	5.4			

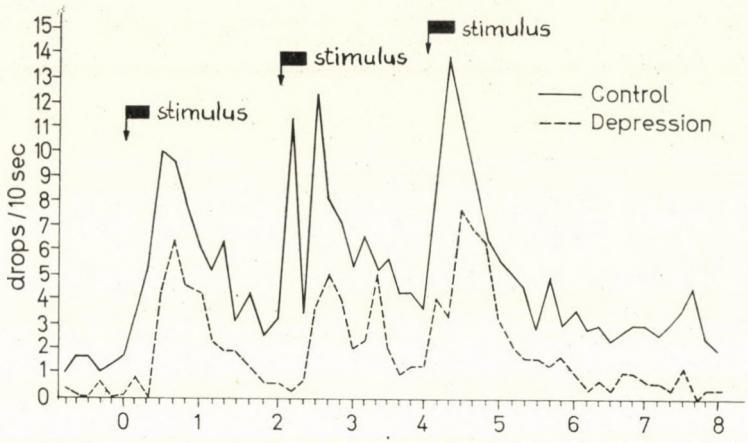

Fig. 7. Parotid response to serial stimulation (three successive citric acid stimuli at intervals of two minutes) plotted against time in the two groups

etc.) have been excluded as far as possible from the present observations, we have none the less to consider dehydration as a potential factor in view of the poor nutrition and fluid intake of depressed patients making them liable to dehydration which may be associated with a reduced salivary secretion. However, in the present cases we failed to detect any sign of dehydration.

b) Parotid secretion was still far from the normal after recovery, when food and fluid intake was no longer inadequate.

c) The haematocrit values estimated in another study (LIPÁK 1971) averaged 44% in the depressed subjects and 42% in the controls. The two figures were practically identical and within the normal range.

On these grounds we feel justified in ruling out the possible role of dehydration in the reduction of salivary secretion.

2. The depressed subjects showed an inverse relationship between the response of parotid secretion and the magnitude of the applied stimulus. The more the stimulus increased, the greater was the divergence between the response of the depressed subjects and of the controls, as illustrated by Fig. 2.

3. Certain facts revealed by the present study seem to account for the contradictions between the observations of GOTTLIEB and PAULSON (1961) and those of DAVIES and PALMAI (1964) in that the poor salivary secretion accompanying the start of the depressive phase, though showing a definite rise parallel with clinical improvement, was still below the control values immediately after recovery.

Table III

Ipsi- and contralateral secretory response of the parotid to hemilateral stimulation with 5% citric acid of the anterior two thirds of the tongue for 20 sec (mean number of drops per min), in the controls (N = 10) and in depressed subjects (N = 10)

Group	N	Drop/min			"Real response" per minute during stimulation				
		before	during stimulation	after	Drop/min	SD	Deviation between the two groups		
							No. of drops	per cent	p
Ipsilateral response									
Control	10	8.9	25.2	14.3	16.3	15.6	−9.9	60	$0.20 > p > 0.10$
Depressed	10	4.8	11.2	3.3	6.4	11.4			
Contralateral response									
Control	10	8.7	16.3	11.3	7.6	7.0	−2,8	36	$0.40 > p > 0.30$
Depressed	10	3.5	8.3	5.4	4.8	5.7			
All responses									
Control	10	8.8	20.5	12.8	11.7	8.8	−6.2	53	$0.10 > p > 0.05$
Depressed	10	4.1	8.6	4.3	5.5	4.5			

Table IV

Secretory response of the parotid to stimulation with 5 ml 5% citric acid for sec ("simultaneous summation"); mean number of drops per min in the controls (N = 15) and in the depressed subjects (N = 20); response of the patiens prior to treatment and after recovery (N = 10)

Group	N	Absolute response, drops/min					"Real" response, drops/min							
		Basal secretion	Stimulation			Global response drops/3′	1′	2′	3′	Global response drops/3′				
										Mean	SD	Deviation between the two groups		
		0′	1′	2′	3′							no. of drops	per cent	p
Control	15	10	44	25	14	83	34	15	4	53	22.5	−16	30	$0.05 > p > 0.02$
Depressed	20	2	24	13	6	43	22	11	4	37	16.9			
Depressed														
before treatment	10	2	21	13	6	40	19	11	4	34	8.1	+13	38	$0.01 > p > 0.001$
after recovery	10	8	37	21	13	71	29	13	5	47	10.9			

Table V

Secretory response of the parotid to two successive stimulations with 5 ml 5% citric acid for 20 sec at an interval of one minute ("successive summation"); mean number of drops per min. in the controls (N = 10) and in depressed subjects (N = 10)

Group	N	Absolute response, drops/min						"Real" response, drops/min								
		Basal secretion	1. Stimulation	2. Stimulation			Global response drops/4′	1. Stimulation	2. Stimulation			Global response, drops/4 min.				
												Mean	SD	Deviation between the two groups		
		0′	1′	2′	3′	4′		1′	2′	3′	4′			no. of drops	per cent	p
Control	10	8.4	33.7	48.5	22.8	18.1	123.1	25.3	40.1	14.4	9.7	89.5	48.0	−42.2	47	$0.05 > p >$
Depressed .	10	1.6	20.7	14.8	11.2	7.0	53.7	19.1	13.2	9.6	5.4	47.3	22.8			0.02

Table VI

Secretory response of the parotid to three successive stimulations with 5 ml 5% citric acid for 20 sec. at intervals of two minutes; mean number of drops per 2 minutes in the controls (N = 10) and in the depressed subjects (N = 10)

Group	N	Absolute response, drops/2min.						"Real" response, drops/2 min.								
		Basal secretion	1. Stimulation	2. Stimulation	3. Stimulation		Global response drops/8′	1. Stimulation	2. Stimulation	3. Stimulation		Global response: drops/8′				
												Mean	SD	Deviation between the two groups		
		0–0′	1–2′	3–4′	5–6′	7–8′		1–2′	3–4′	5–6′	7–8′			no. of drops	per cent	p
Control	10	18.6	66.6	79.1	79.5	34.5	259.6	48.0	60.5	60.9	15.9	185.3	36.9	−95.4	51	$p < 0.001$
Depressed .	10	4.0	29.6	29.0	40.3	7.0	105.9	25.6	25.0	36.3	3.0	89.9	19.6			

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THE ROLE OF LONG-ACTING THYROID STIMULATOR AND OF THYROID AUTOIMMUNITY IN THE PATHOGENESIS OF EUTHYROID ENDOCRINE OPHTHALMOPATHY

By

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The presence of LATS—IgG in euthyroid endocrine ophthalmopathy is confined to the blood plasma of some patients. This is also valid for thyroid antibodies. LATS and thyroid autoimmunity are discussed as possible responsible factors and pointed out as being of secondary nature rather than of causal significance, though the possibility of their involvement in the production of endocrine ophthalmopathy as adjuvant factors is admitted. The present results confirmed the existence of close relationship between the various types of endocrine ophthalmopathy. Observations relative to the transformation of one type into another are interpreted as being conclusive of this fact.

Endocrine ophthalmopathy may occur in hyperthyroid, euthyroid, and even in hypothyroid conditions. Its direct cause is uncertain. In recent years, investigations have been focussed on the long-acting thyroid stimulator (LATS), on thyroid auto-immunity and on the exophthalmos-producing substance (EPS) as possible factors responsible for its production.

Investigations concerned with the aetiological role of LATS and of thyroid autoimmunity in the production of endocrine ophthalmopathy are preferably carried out in euthyroid subjects, hyperthyroidism being often associated with the presence of LATS and of thyroid autoantibodies even in the absence of eye signs. The clinical syndrome of endocrine ophthalmopathy associated with euthyroidism and the accompanying abnormalities of thyroid function were first described by WERNER [42] and termed ophthalmic form of Graves disease. Our observations concerning this question have been reported earlier [14]. Further issues of the problem, namely the possible role of LATS and of thyroid autoimmunity as also their relationships with the results of the triiodothyronine suppression test have been studied and form the subject of the present report.

Material and method

Plasma LATS was estimated by the modified MCKENZIE technique [15, 34]. Analysis of the data obtained was based on geometrical mean. The reaction was considered positive if the mean radioactivity measured in the blood of plasma-treated mice 8 hrs after intravenous administration exceeded 150% of the activity in the controls, i.e. of the group given a 5% albumin solution, the lower fiducial limit being also in excess of 150.

LATS estimation, based on concentration and separation of IgG, was performed by the method of CARNIERO et al. [5, 16] on 36×2.5 cm DEAE-Sephadex-A 50 column with 0.02 M phosphate buffer pH 6.6. The protein fraction containing the pseudoglobulin-IgG served for the assay of LATS.

In three cases the plasma TSH level was also estimated using the concentration procedure of ADAMS and KENNEDY [1, 13]. The TSH-content of plasma extracts was determined in mice; their thyroid-stimulating effect was considered positive if 2 hours after intravenous administration of the preparation the blood activity exceeded the 8-hour value, moreover if mean activity exceeded the mean blood ^{125}I-activity in the control group.

The complement fixation test (CF) for the demonstration of antimicrosomal antibodies and BOYDEN's passive hemagglutination test for thyroglobulin antibodies (TRC) were performed as described earlier [41]. The thyroid antibody assays were considered positive in the case of a CF of 1 : 6 and of a TRC of 1 : 2500 or higher.

The triiodothyronine suppression test was carried out by the estimation of ^{131}I uptake by the thyroid prior to and after administration of 80 μg of l-triiodothyronine daily for six days. To be considered normal, 24-hour iodine uptake had to be at least 40% less after than before the test. Suppression was regarded as disturbed if it failed to attain that value.

The curve of thyroid ^{131}I-uptake, and the values derived from measurements of plasma activity are known to be frequently abnormal in euthyroid endocrine ophthalmopathy [25, 14]. For this reason, in addition to the above assays, the Hamolsky-test was performed in each case and the serum level of protein-bound iodine as well as that of euthyroid endocrine ophthalmopathy, aside from clinical features, the results of all three tests had to be indicative of euthyroidism.

The diagnosis of endocrine ophthalmopathy was based on WERNER's criteria and on the recommendations issued by the American Thyroid Society [43]. On the grounds of this classification, Groups 2 to 6 to be regarded as "progressive" or "malignant" exophthalmos combine the following features: periorbital oedema, exophthalmos, involvement of extraocular muscles, corneal ulceration and impairment or loss of vision as a result of optic nerve lesion. We followed WERNER's classification with the sole difference that we place patients with Hertel-values over 20/100 mm in the group of distinct exophthalmos.

Endocrine ophthalmopathy was present in one of our 12 patients with Hashimoto's disease. Since thyroid biopsy was done in few of them, the diagnosis was made dependent on strict criteria including the presence of euthyroid or hypothyroid goitre, accelerated ESR, positive protein lability tests, increased gamma globulin level, demonstrable thyroid antibodies with a minimum of 1 : 12 for CF and 1 : 25 000 for TRC. If these features were inconsistent, in other words inconclusive of autoimmune thyroiditis, the patients were not included in the Hashimoto-group.

Results

Table I shows the results for all 15 euthyroid subjects with endocrine ophthalmopathy. Those of the female patient L.N. are regarded as being consistent with Hashimoto's disease (ESR 35 mm/hour, thymol turbidity test, 12 U; thymol flocculation test ++++; gamma globulin, 35%; CF, 1 : 48; TRC, 1 : 25,000; serum protein-bound iodine, 3 μg per 100 ml; BE^{125}I, 63%; Hamolsky-test, 11%; serum cholesterol, 256 mg per 100 ml.) This patient, as well as the others were found euthyroid still at the end of the observation period which was not shorter than six months in any of the cases.

From Table II it emerges that upon administration to mice of the non-concentrated plasma of euthyroid subjects with endocrine ophthalmopathy a reaction indicative of LATS was confined to 3 out of 15 cases. After submitting the plasma to the procedure based on separation and concentration of pseudoglobulin-IgG, the extracts yielded positive reactions for LATS in as many as 6 cases. However, even with the aid of the concentration procedure we failed to demonstrate TSH in three of the subjects studied.

Table I

Results of assays for LATS and for thyroid antibodies and of the triiodothyronine suppression test in patients with euthyroid endocrine ophthalmopathy

(Only positive results are shown; suppr. = ^{131}I uptake by thyroid is suppressible by triiodothyronine)

Name	Untreated plasma LATS	LATS—IgG	CF	TRC	Suppression test
L. N.	negative	negative	1 : 48	1 : 250 000	suppr.
Sz. S.	negative	354 (345—364)	1 : 6	negative	suppr.
Sz. L.	negative	negative	negative	negative	not suppr.
S. O.	negative	negative	negative	negative	not suppr.
Gy. E.	negative	negative	negative	negative	not suppr.
O. S.	negative	negative	negative	negative	not suppr.
P. I.	228 (195—268)	222 (210—234)	negative	1 : 2500	not suppr.
V. E.	negative	negative	1 : 12	negative	suppr.
W. I.	negative	218 (208—229)	negative	negative	not suppr.
E. Gy.	180 (168—193)	263 (246—282)	negative	negative	not suppr.
K. A.	450 (392—516)	759 (661—871)	1 : 12	1 : 25 000	not suppr.
D. H.	negative	negative	negative	negative	suppr.
V. E.	negative	negative	negative	1 : 2500	suppr.
Cs. I.	negative	389 (361—418)	negative	negative	not suppr.
Gy. G.	negative	negative	1 : 6	negative	not suppr.

Table II

Plasma LATS and TSH in endocrine ophthalmopathy in euthyroid subjects

Total number of cases	Untreated plasma LATS		LATS—IgG	
	neg.	pos.	neg.	pos.
15	12	3	9	6
	TSH		cc—TSH	
15	15	0	3	0

As it can be seen from Table III, CF was positive in 5, TRC in 4 cases, the values of both being abnormal in two of these patients.

Table IV presents the relationship between the results of the triiodothyronine suppression test and the demonstrability of LATS–IgG. Among the 6 LATS-positive cases there was but one in which it was possible to reduce thyroid ^{125}I uptake by more than 40%, in opposition to the LATS-negative cases where suppression of ^{131}I-uptake was unimpaired in 5 out of 9 patients.

Table III

Thyroid antibodies in euthyroid endocrine ophthalmopathy

CF			TRC		
neg.	1 : 3	≧1 : 6	neg.	1 : 5–1 : 250	≧1 : 2500
9	1	5	6	5	4

It might be of interest to present the case of an originally euthyroid ophthalmopathy in detail where the appearance of hyperthyroidism, together with high initial plasma-TSH levels, replaced later by LATS, was witnessed in the course of the observation period of five years.

The patient was P.A., a 48-year-old female. Early in 1965 she experienced a rapidly progressing protrusion of her right eye associated with impairment of vision, intensive lachrymal secretion, lagophthalmos and conjunctivitis. On admission she appeared euthyroid clinically. The results of investigations relevant to thyroid function were: serum PBJ, 3.2 μg per 100 ml; ^{131}I uptake, 43, 58, 66 and 72%; 48/2 hr plasma test, 0.04; Hamolsky-test, 11%, serum cholesterol, 200 mg per 100 ml; serum TSH, 397% (333—472), i.e. increased; LATS, negative (125%). Ophthalmological findings: Hertel-value, 22—18/98. The diagnosis of euthyroid endocrine ophthalmopathy having been made on these grounds, X-ray treatment was applied to the pituitary, and prednisolone and liothyronine were administered. Two months later the Hertel-value was 20—18/98.

Table IV

Relationship of triiodothyronine suppression test with the plasma LATS level in euthyroid endocrine ophthalmopathy

	Suppression	Failure of suppression
LATS—IgG pos. 6	1	5
LATS—IgG neg. 9	5	4
Total number of cases	6	9

Preseting for control in 1968 she was found euthyroid with occasional elevations of blood pressure. PBJ was 5.1 μg/per 100 ml; the Hamolsky-test, 12.6, serum cholesterol, 225 mg per 100 ml. LATS had become demonstrable in the plasma, its level being 256% (247—265), parallel with the disappearance of TSH (107%). The Hertel-value was 21.5—17/95.

In September, 1968, she had noticed perspirations, tremor, intolerance to heat, palpitations, and had lost 6 kg in weight despite a good appetite. We noted occasional bouts of atrial fibrillation. The values of ^{131}I-uptake were 62, 74, 70 and 62%. The 48/2 hr plasma test was 1.15; serum cholesterol, 143 mg per 100 ml; PBJ in serum, 8.3 μg per 100 ml; Hamolsky test, 20%; plasma LATS, 408% (359—455). The Hertel value was 21.5 — 17/95. This time, the diagnosis was hyperthyroidism with ophthalmopathy. She was given 7000 rad = 6 mCi ^{131}I, followed by thiamazole, prednisolone, Trasicor, and diazepam.

As a result of six months antithyroid therapy she became euthyroid. However, soon after the treatment had been discontinued the hyperthyroid manifestations successively reappeared and increased in severity after an infection of the upper respiratory tract. There was a sudden progression of exophthalmos, particularly on the right side, accompanied by periorbital oedema, increased lachrymal secretion, and lagophthalmos with conjunctivitis. The clinical picture was marked by occasional elevations of blood pressure with general malaise and the paroxysms of atrial fibrillation reappeared. The values for ^{131}I uptake were 72, 81, 67 and 58%; the 48/2 hr. plasma test was 4.2; serum PBJ, 7.4 μg per 100 ml; the Hamolsky test 18%, serum thyroxine, 8 μg per 100 ml. Serum triiodothyronine, expressed in per cents of the total plasma activity, was 8.6%, 48 hrs after the administration of ^{131}I. Plasma LATS was 929% (888—953); CF was negative; TRC was positive, 1 : 2500; the urinary catecholamine excretion was 30 to 40 μg daily. Ophthalmological findings: Hertel-value 28—21/95. Hyperthyroid ophthalmopathy was diagnosed and prednisolone, Thiamazole, Piothyronine radiotherapy and librium were prescribed. Marked improvement ensued but prednisolone had to be discontinued because of gastric complaints. In consequence, the ophthalmic signs greatly deteriorated though she remained euthyroid. In view of a possible autoimmune aetiology, she was put on 100 mg arathioprine daily and small doses of prednisolone on which she has remained since without experiencing any adverse effect. The Hertel value has decreased to 21—17/95.

Discussion

The possibility of LATS being involved in the pathomechanism of euthyroid endocrine ophthalmopathy has closely been studied in recent years but the question is still unsettled. While certain authors incriminate LATS for

the ophthalmic signs [22, 28, 31, 37], others reject this possibility [2, 3, 19, 29, 33, 36]. Clear insight into the problem is made difficult by the circumstance that while LATS occurs in many cases of hyperthyroidism even in the absence of ophthalmopathy, a number of ophthalmic patients, though euthyroid at the time being may have had hyperthyroid episodes which have passed unnoticed.

On the evidence of the present study, blood plasma of the patients with euthyroid ophthalmopathy, which had not been submitted to the concentration procedure, exhibited a LATS-like reaction in only few mice. This might be explained by the low concentration of LATS in the blood of the patients. However, the LATS-like reaction demonstrated with untreated plasma in a few cases, may well be non-specific, as suggested lately by CHOPRA et al. who emphasize the importance of IgG separation [7]. Since no studies of this kind with euthyroid plasma have thus far been reported, we performed parallel assays for LATS in every case with plasma in its untreated state and after the separation and isolation procedure. This allowed to demonstrate LATS in 6 out of the 15 cases. Thus, LATS would seem to be present in approximately one third of the cases, though at low concentrations, whereas in the other two thirds it cannot be detected. On these grounds it seems highly improbable that LATS should by itself be responsible for the exophthalmos even though it may well represent an additional factor, moreover, its presence in one third of the cases clearly points to the close relationships existing between the hyperthyroid and euthyroid forms of endocrine ophthalmopathy.

Some authors explain the existence of euthyroidism in endocrine ophthalmopathy by a reduced "reserve capacity" of the thyroid affecting its responsiveness to LATS-stimulation, the primary cause being attributed to autoimmune thyroiditis [30, 35]. The significance of autoimmune thyroiditis in endocrine ophthalmopathy is consistent with certain observations. There is, for instance, evidence of high-titre thyroid antibodies in 50% of the cases [2, 18, 19] and, according to some observations, endocrine ophthalmopathy may occur in Hashimoto's disease, too [17, 32, 45]. In the present material the frequency of thyroid autoimmunity was not greater than in hyperthyroid patients in general [12], nor were the antibody-titres higher. This would therefore be at variance with the possible aetiologic involvement of autoimmune thyroiditis. On the other hand, among our 12 cases of Hashimoto's disease we had one patient with endocrine ophthalmopathy — in whose blood LATS was absent. According to the present results, thyroid autoimmunity seems to play no decisive part in the production of endocrine ophthalmopathy. However, the fact that it does none the less occur in a number of cases points to close relationships between the individual types of thyroid disease on the one hand, while being suggestive, in the other, of the involvement of some aetiological factor which may possibly result in thyroid autoimmunity.

In euthyroid endocrine ophthalmopathy, similarly to hyperthyroidism, triiodothyronine fails to suppress ^{131}I uptake by the thyroid. This abnormal reaction has been noted with a variable frequency [32, 4, 19, 30, 42, 45], probably in accordance with the selection of the given material. The pathomechanism of the abnormal triiodothyronine suppression test has yet to be clarified. The involvement of LATS is highly problematic, and the latest observations relative to cases of cured thyrotoxicosis are likewise contradictory [8, 9, 20, 21, 40]. It seemed therefore of interest to examine these relationships in the euthyroid forms of Graves' disease. The results indicated that in this syndrome production of an abnormal suppression test is not necessarily linked to the presence of LATS and, on the other hand, the triiodothyronine suppression test may occasionally be abnormal in LATS-positive cases.

No proof could be more conclusive of the close connections between euthyroid and hyperthyroid endocrine ophthalmopathy than observations of their transformation into one another. This gives particular interest to the case of patient P.A. reported in this study. In the course of a long follow-up period the originally euthyroid patient became hyperthyroid; at the same time TSH, initially of high concentration, disappeared from the plasma and was replaced by LATS, the values of which greatly increased after ^{131}I treatment, parallel with a progression of the ophthalmic signs. Transformation of euthyroid into hyperthyroid endocrine ophthalmopathy has been witnessed by other authors as well [26] and attributed to the close relationships between the two forms.

The high plasma TSH-level in patient P.A. in the early stage of the disease raises the possibility that the beginning of the process may be associated with an increased production of thyrotrophic hormone which later gradually ceases. In endocrine ophthalmopathy the TSH-level is not higher than under normal conditions, as confirmed by three of our cases where even with the aid of the concentration procedure we failed to demonstrate an increased plasma TSH level. Our observation of increasing LATS-levels together with a deterioration of the ophthalmic signs consequent upon radioiodine therapy is in accordance with other data [27] and it is on these very grounds that KRISS [28] attributes a role to LATS and to autoimmunity in the pathogenesis of endocrine ophthalmopathy. Observations of this kind have, however, been not uniformly confirmed [4] and even in our material they were confined to two cases.

Time and again, the pathogenetic significance of EPS is being dealt with in the literature [23, 28]. This substance has not yet been identified, though it seems to be distinct from TSH as well as from LATS [11, 24]. The fact that the effect of EPS can be best demonstrated in fish is a major source of difficulties. The substance has been found by WINAUDÉ et al. [44] to be like-

wise an IgG without being identical with LATS and, according to DANDONA and EL KABIR [10] this IgG, unlike LATS, has the capacity of increasing the weight of the Harderian gland, an intraorbital structure in the guinea-pig. This observation calls for further studies.

It has been ascertained in the present study that in euthyroid endocrine ophthalmopathy, despite a clinically normal thyroid function and normal hormonal iodine levels in the blood, LATS and thyroid autoimmunity are demonstrable in a number of cases and the triiodothyronine suppression test also yields abnormal values, thus revealing disorders in thyroid activity. However, neither LATS nor thyroid autoimmunity seem to be responsible for the development of endocrine ophthalmopathy. It is far more likely that they are secondary to the process though they might still play some part in its production. From our results it further emerges that the presence of LATS does not necessarily result in an enhanced thyroid function even when the co-existence of an immune thyroiditis destructive to the thyroid substrate can be ruled out. On these grounds, the aim of further studies is to find the substance responsible for the ophthalmopathy as well as for the secondary changes. The present study has furnished evidence in support of the close relationships existing between the individual types of endocrine ophthalmopathy, its transformation into its hyperthyroid form as well as the occurrence of ophthalmopathy in Hashimoto's disease, even though rare, are particularly suggestive.

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RENIN ACTIVITY OF RENAL VENOUS BLOOD IN EXPERIMENTAL HYPERTENSION INDUCED BY LIGATION OF ONE RENAL ARTERY IN DOGS

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Development of arterial hypertension in its acute and chronic phases has been studied in dogs after the ligation of one renal artery with the other kidney unaffected. It has been demonstrated on the grounds of renin activity in the venous blood of both the ischaemic and the unaffected kidney as well as in peripheral blood, that a pressor substance is being produced and released into the circulation by the ischaemic, and after its removal by the contralateral kidney. While the production of hypertension was due to the pressor agent secreted by the ischaemic kidney, its persistence after removal of the affected organ was the result of continued secretion of the substance by the unoperated kidney.

FAHR (1925) was the first to point to a possible involvement of renal ischaemia in the induction of arterial hypertension, a postulate borne out later by the experiments of HARTWICH (1929) and of GOLDBLATT et al. (1934) in which hypertension was induced in dogs by unilateral constriction of the renal artery combined with removal of the other kidney or by the constriction of both renal arteries. On the evidence of these studies it has been universally accepted that disturbances of intrarenal haemodynamics due in many cases to a narrowing or obstruction of the renal artery or of its branches, result in the clinical syndrome of renovascular hypertension.

While PICKERING and PRINZMETAL (1937) induced arterial hypertension in rabbits by similar means as the last-named authors, WILSON and BYROM (1939) found that the unilateral intervention sufficed for bringing about a hypertensive state in rats. In this manner the various research teams studying the mechanism of renovascular hypertension had adequate models on different species at their command. The finding of PAGE (1935) and of FREEMANN and PAGE (1937) that denervation, transplantation or sympathectomy failed to arrest the development of renovascular hypertension brought the issue of a possible humoral mechanism into focus and initiated a search for the potential pressor agent. Then PAGE (1936), BRAUN-MENENDEZ et al. (1940), PAGE and HELMER (1940) in fact observed that constriction of the renal artery results in the release into the blood stream of a pressor substance which produces arterial hypertension by its constrictive effect on the smooth muscle elements of the arteriolar wall. These findings indicated that it was the produc-

tion of renin by the ischaemic kidney which accounted for experimental renal hypertension.

GOLDBLATT pointed out as early as 1937 that in the presence of an unaffected kidney, experimental hypertension in dogs, if being produced at all, is only transitory. PAGE (1941) reported on the favourable effect of normal kidney extract in hypertensive disease, presuming that the normal kidney either destroys the pressor substance or produces some factor of antipressor activity.

The rat-model employed by WILSON and BYROM (1939) in which one kidney had been left unaffected, furthermore those acute dog experiments in which one kidney was kept under a high and the other under a low blood pressure by the aid of a clamp placed between the two renal arteries, provided for the parallel study of the ischaemic and the unmanipulated kidney in the same animal. On the evidence of these experiments, it has been found that renin concentration is increased in the ischaemic, and reduced in the unmanipulated contralateral kidney (TOBIAN 1962, SOKABE and GROLLMAN 1963).

It has been shown earlier that a total ligation of one renal artery in dogs with their other kidney unaffected is followed in a few days by the appearance of arterial hypertension which subsequently becomes chronic (FEKETE 1967). This hypertensive state may be regarded on clinical and morphological grounds as an experimental model of renovascular hypertension (FEKETE 1970, FEKETE et al. 1971), the vasoconstriction due to renin release suggesting itself as the most likely pathogenetic factor. In the present paper the results of further studies concerned with the mechanism of production and maintenance of acute and chronic hypertension induced by constriction of one renal artery in dogs will be reported.

Material and methods

In 16 adult mongrel dogs of either sex under pentobarbital anaesthesia the left (in some cases the right) renal artery and, if present, the supernumerary renal branches of the aorta, were ligated close to their origin from the paracostal approach under aseptic conditions, and the wound was closed. Arterial blood pressure was checked prior to and at regular intervals after the intervention. Mean arterial pressure was measured percutaneously in the femoral artery by a mercury manometer in alert animals which had been habituated to the intervention. For the estimation of pressor activity, blood samples were withdrawn from the veins of both kidney and from peripheral blood at determined intervals after the intervention (day 0). The procedure was as follows: The animals were anaesthesized with pentobarbital and peripheral blood samples were withdrawn from the femoral artery by needle puncture. Subsequently, the abdomen was opened under aseptic conditions, and samples of venous blood of the kidney with its artery constricted were withdrawn, then this kidney was removed, weighed and preserved for microscopic study. Then venous blood was withdrawn from the unoperated kidney, too, either by needle puncture or via a catheter inserted through the femoral vein, care being taken in both cases to obtain strictly venous blood for the measurements, and then the abdominal wound was closed.

In the further course of the study, blood pressure was measured at 10 to 20-day intervals by the technique referred to earlier and samples were taken from the venous blood of the unoperated kidney and from the peripheral blood of the same animal at determined times for the

estimation of pressor activity. Between the 60th and 570th day after ligation the remaining kidney was removed, weighed and preserved for microscopic study.

Before the blood samplings, in three dogs the venous blood of the unmanipulated kidney was diverted into the jugular vein with the aid of a plastic T-tube allowing a direct determination of renal blood flow. The same tube was used for blood samplings for estimation of pressor activity.

Estimation of pressor activity

For the estimation of plasma renin activity the method of KANEKO et al. (1967) was used in a slightly modified form. 20 ml blood taken from the femoral artery or from the renal vein was transferred into a plastic tube containing 0.05 g EDTA and centrifuged immediately at 3000 r.p.m. at 4° C for 10 min. The plasma was stored for 16 hours at 0° C, then its pH being adjusted to 5.5 and neomycin being added, it was incubated at 37° C for 24 hours, then boiled so as to stop the enzyme reaction, and the pH of the protein-free filtrate was adjusted to 7.4. Plasma pressor activity was titrated by comparing it with that of a known amount of Hypertensin (Ciba) in rats of 200 to 250 g body weight of our own stock, having been nephrectomized 24 hours before the experiment, pretreated with pentholinium, anaesthesized with pentobarbital and vagotomized. Renin activity was expressed in terms of angiotensin II equivalent ng/ml/24 hrs. By the procedure in 42 instances from 18 to 37% of the initial amount was gained back and the difference between two parallel measurements was 9.98 ± 9.41. On the evidence of 22 estimations, renin activity in the peripheral blood of a dog with normal blood pressure and normal plasma sodium was 12.2 ± 9.8 ng/ml/24 hrs.

The results were evaluated by Student's two-sample t test.

For the histological studies the removed kidneys were fixed in 6% formaldehyde. Then specimens were taken from three different areas of the kidney and stained with the Mallory—Farkas, Mallory—Endes, azan, and Weigert's resorcin-fuchsin dyes.

Results

Table I presents the data for 16 dogs in which hypertension has been induced by ligation of one renal artery. Column I shows the serial number, body weight and sex of the animals. In the second vertical column the results are numbered as follows: 1. arterial blood pressure, 2. renin activity of the venous blood of the unoperated kidney, 3. renin activity in peripheral blood, and 4. renin activity in the ischaemic kidney. The further columns show the data obtained at 10—20, 40, and 100 days. On day 0, measurement of the original (normal) blood pressure and ligation of the left or right renal artery (LL and RL) were performed. Pressor activity was estimated in the same dog on 3 to 6 occasions. It is seen from Table I that the periods of observation were not identical, six dogs having been observed for 60 to 140 days, seven dogs for 220 to 320 days and three dogs for 560 days. Blood pressure was checked even beyond this period in several animals. The ischaemic kidney was removed in the state of hypertension (LN = left nephrectomy, RN = right nephrectomy).

It has been found that

1. *arterial blood pressure*, compared with the control value of 126 ± 4 mmHg, rose after renal artery ligation and remained significantly higher during the entire observation period (comparison of the values of the individual vertical columns with the normal blood pressure values: $p < 0.001$ and $p < 0.01$, respectively),

Table I

Arterial blood pressure and renin activity in dogs with renovascular hypertension

Animal	Test	0	20–30	31–40	41–50	51–60	61–70	71–90
132/1968	1	140	190	160	170	170	—	170
20 kg ♂	2	—	—	—	—	42	—	—
	3	—	128	—	—	22	—	—
	4	—LL	184 LN	—	—	—	—	—
119/1968	1	135	180	160	170	170	—	170
17 kg ♂	2	—	—	—	—	20	—	—
	3	—	200	—	—	8	—	—
	4	—LL	320 LN	—	—	—		—
135/1968	1	120	160	170	170	—	—	170
14 kg ♂	2	—	—	—	—	—	—	—
	3	—	—	—	—	—	—	—
	4	—LL	—LN	—	—	—	—	—
131/1968	1	140	170	190	180	160	—	—
16 kg ♂	2	—	—	300	—	—	—	—
	3	—	—	80	—	—	—	—
	4	—RL	—	160 RN	—	—	—	—
121/1968	1	120	170	190	170	170	160	190
16 kg ♂	2	—	—	540	—	—	—	—
	3	—	—	480	—	—	—	—
	4	—LL	—	380 LN	—	—	—	—
74/1970	1	130	190	185	—	—	180	180
17 kg ♀	2	—	—	—	—	—	180	41
	3	—	—	0	—	—	24	0
	4	—LL	—	41 LN	—	—	—	—
77/1970	1	130	210	220	—	—	—	—
17 kg ♀	2	—	—	36	—	—	—	—
	3	—	—	25	—	—	—	—
	4	—LL	—	36 LN	—	—	—	—
31/1970	1	130	170	—	165	—	—	175
12 kg ♀	2	—	—	—	28	—	—	42
	3	—	—	—	14	—	—	11
	4	—RL	—	—	16 RN	—	—	—

LL = complete ligation of left renal artery; RL = complete ligation of right renal artery
LN = left nephrectomy; RN = right nephrectomy

during a period of observation extending to 570 days

91—100	101—120	121—140	141—160	161—180	200—220	221—240	241—260	320—420	560—570
170	160	—	170	170	190	180	170	—	—
—	45	—	—	92	—	25	50	—	—
—	32	—	—	56	—	17	11	—	—
—	—	—	—	—	—	—	—	—	—
170	160	—	170	160	190	190	170	—	—
—	38	—	—	170	—	—	112	—	—
—	27	—	—	118	—	—	78	—	—
—	—	—	—	—	—	—	—	—	—
—	180	—	175	—	170	—	—	170	—
—	—	—	—	—	—	—	—	110	—
—	—	—	—	—	—	—	—	67	—
—	—	—	—	—	—	—	—	—	—
160	160	—	160	—	180	170	180	—	—
42	—	—	360	—	—	34	17	—	—
20	—	—	120	—	—	32	8	—	—
—	—	—	—	—	—	—	—	—	—
165	170	—	180	170	180	185	180	—	—
38	—	—	62	—	—	24	28	—	—
5	—	—	60	—	—	22	28	—	—
—	—	—	—	—	—	—	—	—	—
—	—	180	—	175	180	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	165	170	—	—	180	—	180	—	—
—	125	330	—	—	—	—	—	—	—
—	30	16	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—

Table I (cont.)

Animal	Test	0	20–30	31–40	41–50	51–60	61–70	71–90
46/1970	1	115	170	—	170	—	—	150
12 kg ♂	2	—	—	—	42	—	—	—
	3	—	—	—	42	—	—	—
	4	—RL	—	—	47 RN	—	—	—
48/1970	1	125	200	—	180	180	—	—
15 kg ♀	2	—	—	—	92	—	—	—
	3	—	—	—	0	—	—	—
	4	—RL	—	—	40 RN	—	—	—
80/1970	1	125	170	160	160	—	180	—
14 kg ♀	2	—	—	195	88	—	—	—
	3	—	—	2	18	—	—	—
	4	—LL	—	35 LN	—	—	—	—
82/1970	1	130	180	—	180	—	160	—
12 kg ♂	2	—	—	—	—	—	—	—
	3	—	—	—	—	—	—	—
	4	—RL	—	—	—	—	—RN	—
227/1968	1	115	170	190	—	—	180	—
12 kg ♂	2	—	—	—	—	—	—	—
	3	—	—	—	—	—	—	—
	4	—LL	—	—	—	—	—	—
221/1968	1	120	160	175	—	—	170	—
11 kg ♂	2	—	—	—	—	—	—	—
	3	—	—	—	—	—	—	—
	4	—LL	—	—	—	—	—	—
49/1969	1	125	190	—	190	175	—	190
15 kg ♀	2	—	—	—	—	—	—	—
	3	—	—	—	—	—	—	—
	4	—LL	—	—	—	—	—	—
53/1969	1	120	180	—	170	175	—	180
14 kg ♀	2	—	—	—	—	—	—	—
	3	—	—	—	—	—	—	—
	4	—LL	—	—	—	—	—	—

91–100	101–120	121–140	141–160	161–180	200–220	221–240	241–260	320–420	560–570
180	170	—	—	175	—	170	170	—	—
42	40	—	—	—	—	—	—	—	—
17	14	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
170	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
160	170	165	—	165	170	—	—	—	—
82	113	230	—	83	220	—	—	—	—
53	82	43	—	29	31	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	170	—	—	175	180	190	—	180	175
—	—	—	—	—	—	—	—	—	130
—	—	—	—	—	—	—	—	—	40
—	—	—	—	—LN	—	—	—	—	—
—	170	—	—	—	175	170	180	180	170
—	—	—	—	—	—	—	—	—	26
—	—	—	—	—	—	—	—	—	0
—	—	—	—	—	—	—	—LN	—	—
—	—	180	—	—	180	—	—	180	—
—	—	—	—	—	—	—	—	38	—
—	—	—	—	—	—	—	—	0	—
—	—	—	—	—	—	—	—	42 LN	—
—	—	180	—	—	—	175	—	175	170
—	—	—	—	—	—	—	—	127	105
—	—	—	—	—	—	—	—	30	47
—	—	—	—	—	—	—	—	145	65 LN

2. *in the venous blood from the ischaemic kidney*, pressor activity was excessively high in four dogs (132, 119, 131, and 121), the mean being 261 ng/ml; lower in six dogs (74, 77, 31, 46, 48 and 80), with a mean of 36 ng/ml. The quotient of renin activity of the venous blood of the ischaemic kidney and that of peripheral blood was 1.89 ± 1.32 ($n = 8$),

3. renin activity in the blood from the *unoperated kidney* was higher than the control value of 12.2 ± 9.8 ng/ml and also than that of peripheral blood. Renin activity in the venous blood of the unoperated kidney was higher than in the ischaemic kidney also immediately after removal of the latter (e.g. in dogs 131, 121, 48, 80 etc.),

4. after *removal of the ischaemic kidney*, renin activity in the venous blood of the unoperated kidney persisted at the increased level (dogs 132, 46, 53) or continued to rise (dogs 119, 31, 82). In two cases, renin activity in renal venous blood was found to correspond to that in peripheral blood but even here it was higher than the normal value. In some dogs the pressor activity of the unoperated kidney could be assessed on a single occasion only; it exceeded the control value (12.9 ± 9.8 ng/ml). The quotient of renin activity in the venous blood of the unaffected kidney and in peripheral blood was 2.71 ± 1.92 ($n = 33$).

One of the illustrative experiments is shown in Fig. 1 in the form of a diagram presenting the data relative to dog 119. Pressor activity was estimated in the venous blood of the ischaemic kidney and in peripheral blood on the 25th day after ligation of the left renal artery. During the hypertensive state, blood samples were obtained from the vein of the unmanipulated kidney on four further occasions and pressor activity was found to exhibit a rising tendency, and to be higher than in peripheral blood. In this dog the full period of observation was 241 days.

In three dogs the observation was completed by an acute experiment consisting in a direct measurement of renal blood flow with the animal under general anaesthesia. In dog 119, the blood flowing through the unoperated right kidney on the 241st day of hypertension was 454 ml/min/100 g renal tissue (renin activity, 112 ng/ml); in dog 227, RBF, measured on the 564th day of hypertension was 631 ml/min/100 g renal tissue (renin activity, 130 ng/ml); and in dog 221, on the 557th day of hypertension it was 571 ml/min/100 g renal tissue (renin activity, 26 ng/ml).

Kidney weight at the end of the experimental period revealed the following relationships (Tables II and III).

1. The ischaemic kidney removed 20 to 40 days after the intervention ("early nephrectomy") displayed extensive atrophy, its weight corresponding to 41% of that of a normal kidney. (Normal renal weight was calculated on the basis of the formula $y = 12 + 4.33$ kg body weight, where y represents total renal weight, i.e. the weight of both kidneys in a dog of a given body

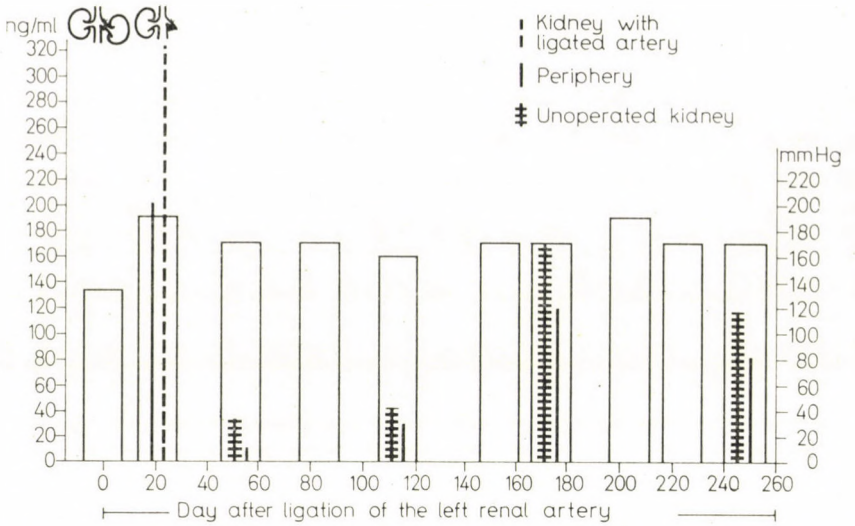

Fig. 1. Dog 119/1968. Day 0: reading of blood pressure and ligation of left renal artery. Day 20: checking of blood pressure and blood sampling for estimation of renin activity. Renin activity in blood of the femoral artery, 200 ng/ml; in the venous blood of the kidney with ligated artery, 320 ng/ml; arterial blood pressure, 180 mmHg. In the further part of the diagram, blood pressure values determined at regular intervals and renin activity estimated on four occasions during the observation period of 241 days are presented. The continuous line represents renin activity in peripheral blood, the interrupted line that in the venous blood of the ischaemic kidney, and the cross-hatched line that in the venous blood of the unoperated kidney

weight (Table II). The unoperated kidney, removed on the 100th to 300th day, was not hypertrophic, its weight practically corresponded to the normal value calculated for one kidney from the total renal weight of the normal animal (98%).

2. The ischaemic kidney removed beyond the 40th day after the intervention ("late nephrectomy") showed advanced atrophy. Its weight was not more than 17% of the normal value (Table III). The contralateral, unoperated kidney removed on the 100th to 300th day was not hypertrophic and ite weight practically corresponded to that of a normal kidney calculated from the total renal weight of normal animals (102%).

3. If during the entire hypertensive state neither of the kidneys had been removed, in other words, if no preliminary nephrectomy of the ischaemic kidney had taken place (dogs 49 and 53), the kidney with the ligated artery underwent severe atrophy to 15.5% of the calculated normal weight, and the unoperated kidney also showed some involution, its actual weight corresponding to 80% of the calculated normal weight.

Table II

Kidney weight in hypertension induced by ligation of one renal artery, in case of removal of the ischaemic kidney 20 to 40 days after the intervention ("early nephrectomy")

No. of animal 1.	Weight of animal, kg 2.	Weight of kidney with ligated artery, g 3.	Weight of unoperated kidney, g 4.	Duration of hypertension (days) 5.	Weight calculated for one kidney in the normal animal, g 6.	Columns 4/6	Columns 3/6
132/1968	20.0	19.0	43.2	243	49.2	0.88	0.39
119/1968	17.0	23.8	48.6	241	42.7	1.14	0.56
135/1968	19.0	24.7	42.8	326	47.2	0.91	0.58
131/1968	16.0	14.0	47.5	248	40.7	1.17	0.34
121/1968	16.0	13.8	38.0	250	40.7	0.93	0.33
74/1970	17.0	15.0	34.2	211	42.7	0.80	0.35
77/1970	17.0	14.1	43.6	150	42.7	1.02	0.33
80/1970	14.0	11.8	32.4	130	32.0	1.01	0.37
$\Sigma x = 136.0$		136.2	330.3		337.9	7.86	3.25
$n = 8$		8	8		8	8	8
$\bar{x} = 17.0$		17.0	41.3		42.2	0.98	0.41
$s_{\bar{x}} = 0.7$		1.7	2.1		1.8	0.05	0.04

Table III

Kidney weight in hypertension induced by ligation of one renal artery, in case of removal of the ischaemic kidney beyond the 40th day after the intervention ("late nephrectomy")

No. of animal 1.	Weight of animal, kg 2.	Weight of kidney with ligated artery, g 3.	Weight of unoperated kidney, g 4.	Duration of hypertension (days) 5.	Weight calculated for one kidney in the normal animal, g 6.	Columns 4/6	Columns 3/6
31/1970	12.0	5.6	34.6	260	32.0	1.08	0.18
46/1970	12.0	8.2	40.0	256	32.0	1.25	0.26
48/1970	15.0	7.4	30.2	140	38.5	0.78	0.19
82/1970	13.0	5.8	35.5	218	34.2	1.04	0.17
227/1968	12.0	4.8	31.5	564	32.0	0.98	0.15
221/1968	11.0	2.5	29.6	557	29.8	0.99	0.08
$\Sigma x = 75.0$		34.3	201.4		198.5	6.12	1.03
$n = 6$		6	6		6	6	6
$\bar{x} = 12.5$		5.7	33.5		33.1	1.02	0.17
$s_{\bar{x}} = 0.6$		0.8	1.7		1.2	0.06	0.03

Histological findings

1. Kidneys with ligated artery showed focal necrosis alternating with atrophic and relatively intact areas. The juxtamedullary glomeruli preserved in the necrotic and atrophic areas, as well as the basement membranes of Bowman's capsule were thickened, with a destruction of the medulla pertaining to these cortical elements. The walls of the blood vessels within the necrotic and atrophic areas displayed hypoxic changes. The size of the unaffected areas varied in the individual animals but it amounted in no instance to more than 10% of the original renal substance in its unaffected state. In these areas the parenchyma was fairly well-preserved, with a slight fibrous proliferation and thickening of Bowman's capsule. The vascular changes in the intact areas corresponded to those exhibited by the unoperated kidney and the changes developing in the two kidneys showed a parallelism in respect of pattern and degree (Fig. 2).

2. In the unoperated kidney, there were two types of alteration. *a)* Slight vascular changes affecting the interlobular and afferent arterioles (swelling of the media and of the internal elastic membrane). Patterns of this kind are usually seen in the early stage of hypertension. *b)* Vascular lesions of major severity: proliferation of the intima, vacuolation of the media, dissociation of the internal elastic membrane, sclerosis of the interlobular and grade I arteries, fibrosis of the afferent arterioles, atrophy of the glomerular loops, protein in Bowman's capsule, thickening of the basement membrane, and frequently a fibrous hyperplasia extending to the renal capsule and producing grooves on the surface of the organ. These features were typical of the kidneys of animals with long-standing hypertension (Fig. 3).

Discussion

In the present experiments, chronic hypertension was induced in dogs by complete ligation of one renal artery, leaving the other kidney unaffected. The hypertensive state induced by unilateral intervention has been studied for periods extending to one or two years (FEKETE, 1967, 1970, 1971), and the following facts have been established.

1. Removal of the ischaemic kidney 20 days after obstruction of its arterial blood supply failed to normalize blood pressure. 2. A certain impairment ensued in some parameters of renal function, for instance the glomerular filtration rate decreased. 3. In the hypertensive state the serum sodium level was unaffected, and basal (spontaneous) sodium excretion did not increase. 4. Water loading was followed by fluid retention and salt loading elicited an increased excretion of sodium. 5. The arterioles of the unaffected kidney underwent changes of the hypertensive type. On the grounds of these features

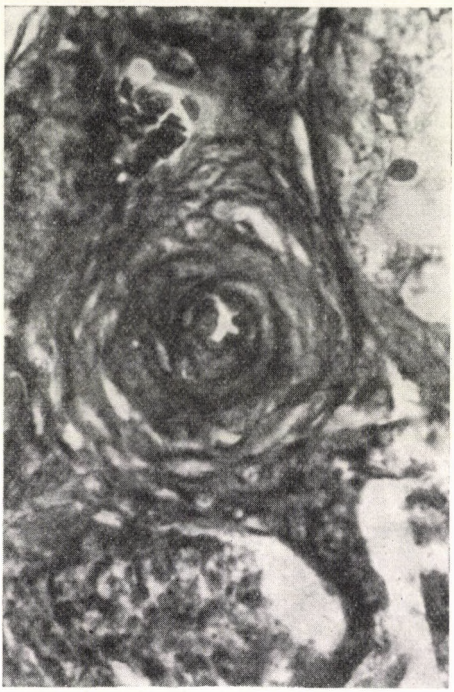

Fig. 2. Dog 77/1970. Kidney after ligation of its artery. In the fairly preserved parenchyma there is a tertiary interlobular arteriole with gross thickening and fibrous hyperplasia of its wall and a narrowed lumen. Azan stain, ×240

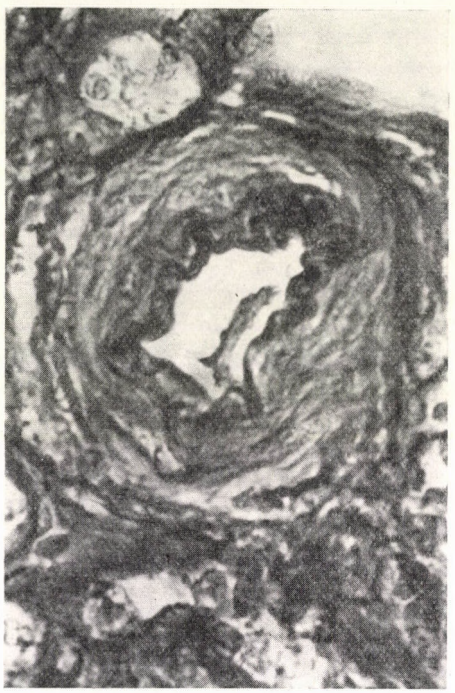

Fig. 3. Dog 77/1970. Unoperated kidney. Primary interlobular artery with fibrous hyperplasia of its wall and dissociation of the internal elastic lamina. Azan stain, ×240

the hypertensive state induced by unilateral intervention in the dog may be regarded to correspond to human renovascular hypertension.

The underlying mechanism of this kind of a hypertensive state involved some controversial questions. Since GOLDBLATT et al. (1934) had succeeded in inducing arterial hypertension by reducing renal flow and in preventing the condition by the simultaneous ligation of the renal vein, the idea of a pressor agent being produced by the ischaemic kidney and released into the blood stream has gained general acceptance and has been confirmed by numerous authors (PAGE 1936, PAGE and HELMER 1940, PICKERING and PRINZMETAL 1942, FLOYER 1957, HELMER 1962, FRANK 1963, SOKABE and GROLLMAN 1963, BARRACLOUGH et al. 1965, BORKOWSKI et al. 1965, MCPHAUL et al. 1966). Then an increase of renin activity was shown in the ischaemic kidney and its venous blood, in contrast to the contralateral (normotensive) kidney in which renin activity was reduced (REGOLI et al. 1962, BATH et al. 1966, HAAS and GOLDBLATT 1959, PRINZMETAL and FRIEDMAN 1936, BOR-

KOWSKI et al. 1965, CARPENTER et al. 1961). As pointed out by VANDER (1967), for a rough quantitative estimate of renin production by the kidney we must know, in addition to the veno-arterious renin difference of the kidney also the renal plasma flow. Since such data are scarce, most studies have been confined to the demonstration of renin-like factors in peripheral blood, in renal venous blood, and in the lymph.

It has been suggested that the part played by the increased secretion of renin was only to give rise to hypertension (DEXTER and HAYNES 1946, LEE 1969). Recently, MACDONALD et al. (1970) have succeeded in inducing hypertension of the Goldblatt-type in rabbits immunized against angiotensin II, moreover FUNDER et al. (1970) found the renin-activity of the renal venous blood of sheep with Goldblatt-hypertension to be unrelated to the level of blood pressure. On the grounds of these findings the authors referred to above reject the possibility of the renin-angiotensin system being involved either in the production or in the maintenance of renovascular hypertension.

GOORMAGHTIGH (1940) suggested the granular cells in the wall of the preglomerular arteries (JGA) as the site of origin of the pressor agent and BOHLE et al. (1953) in fact found an increased granulation in rat kidneys with a restricted blood supply. The JGA-granules were noted by TOBIAN et al. (1958) to double their number in the ischaemic kidney in hypertension induced in rats by constriction of one renal artery, while the granules practically vanished from the unmanipulated kidney. Correlations between the JGA-granulation and the amount of renin extractable from the kidney have been demonstrated by TOBIAN (1962), in agreement with PICKERING's statement (1955) that the presence of renin in the kidney is confined to the glomerular areas.

The aim of the present experiments was to throw light on the mechanism giving rise to and maintaining the arterial hypertension resulting after the total constriction of one renal artery. The information provided by the results of the study fall into two parts.

1. In the venous blood of the ischaemic kidney a high renin activity was demonstrable. This may be interpreted as follows. The possibility of renin production is given in the ischaemic kidney since after ligation of its artery a collateral circulation is readily formed (FEKETE 1967). On the evidence of our estimations, the collateral system thus produced ensures 1/10 to 1/4 of the original blood supply to the kidney and primarily concerns its cortical, thus its renin producing, areas. This is consistent with histological evidence in that the intact areas of the kidney may be estimated at 10% of the original renal substance. The pressor agent thus finds unhindered and continuous access to the blood stream via the renal vein. To quote a few illustrative figures, the kidneys of dogs 132 and 119 weighed 19.0 and 23.8 g, respectively, on the 25th day after ligation, thus at the time of blood samplings and nephrectomy.

As it has been shown earlier, with a similar renal weight the absolute value for renal blood flow ranges between 15 and 20 ml/min and, referred to 100 g renal tissue, it would correspond to approximately 80 to 90 ml/min. Secretion of renin by the ischaemic kidney thus doubtlessly constitutes the primary factor in the arisal of hypertension induced by unilateral constriction of the renal artery in dogs.

2. After the ischaemic kidney had been removed, the hypertension was none the less found to persist over long periods. This observation gives inevitably rise to the question, what kind of a mechanism may maintain the hypertensive state when renin production by the ischaemic kidney has ceased.

a) The blood from the contralateral, unoperated, kidney revealed a high renin activity, this either showed a rising tendency or its level was stable during the whole hypertensive period, but in all instances significantly above the control value. At the same time, the serum sodium level remained in its normotensive range, corresponding to 150 ± 4 mEq/l in both cases. Neither did the NPN show an increase, and proteinuria was absent.

b) Under normal conditions (ADDIS 1924), after unilateral nephrectomy the weight of the remaining kidney gradually attains 75% of the original total renal weight. The process of hypertrophy starts together with the loss of renal parenchyma and attains its maximum by the end of the 20th day (ROLLASON 1949). The increase in renal tissue mass results from a tubular hyperplasia and hypertrophy and from glomerular swelling (ARATAKI 1926).

As pointed out earlier, there is ample evidence to show that the contralateral kidney is in fact far from being unaffected, as clearly shown by its impaired functions, inadequate adaptation and morphological changes. The present study has brought to light an additional feature, the lack of a compensatory renal hypertrophy in the hypertensive state. In fact, the mass of the remaining kidney was not greater than it would be in the presence of a normally functioning other kidney.

c) In the chronic hypertensive state, renal blood flow was found to be normal. Since on the evidence of the microscopic findings there was a narrowing of the interlobular arterioles, there can be little doubt about the existence of an increase in preglomerular resistance. (The systemic and intrarenal haemodynamic parameters in dogs with renovascular hypertension form the subject of another paper (FEKETE and FAZEKAS 1971). Therefore, despite the elevated blood pressure it seems most likely that the pressure prevailing in the afferent arterioles is not significantly increased, owing to the sclerotic rigidity of the vascular wall. In this manner the transmural pressure increasing effect of hypertension fails to assert itself adequately, if at all, in the afferent arterioles, and, in the absence of the necessary impulses to the stretch-receptors, a hypersecretion of the juxtaglomerular cells will ensue as an inevitable course of events.

d) If it is true that, as suggested by the results of VANDER (1967), FUNDER et al. (1970), WATHEN et al. (1965), BUNAG et al. (1968), in addition to intrarenal mechanisms, extrarenal factors (sympathetic innervation, catecholamines) are also involved in the chronic phase of renovascular hypertension, then the release of renin is most certainly enhanced by the increase in arteriolar tone due to the increased sympathetic innervation (VANDER 1965).

Thus, the arisal of renovascular hypertension may be ascribed to the secretion and release of a pressor substance by the ischaemic kidney. This factor increases intrarenal vascular resistance and gives thereby rise to degenerative changes in the arterioles. The hypertensive state persisting after removal of the ischaemic kidney may have the production of pressor substance by the contralateral, the unmanipulated, kidney as its possible mechanism. While a compensatory capillarization and hypertrophy of the contralateral kidney is inhibited by the arteriolar sclerosis involving the organ, JGA hypergranulation ensues due to the rigidity and narrowing of the arterioles. The pressor substance produced by the "unaffected" kidney has been demonstrated in its venous blood.

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EFFECT OF ORAL ANTIDIABETIC AGENTS ON DIURESIS IN DIABETES INSIPIDUS OF THE VASOPRESSIN-RESPONSIVE AND THE NEPHROGENOUS TYPES

By

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Oral antidiabetic agents, including buformin, chlorhexamide and chlorpropamide were administered to five subjects with vasopressin-responsive, and two with nephrogenic diabetes insipidus and the parameters of urinary output were registered. Chlorpropamide was found to produce a significant fall in urinary output and free water clearance with an increase in urinary osmolality in four of the patients with ADH-responsive diabetes insipidus, whereas buformin and chlorhexamide failed to influence the water-balance to any significant extent. Diabetes insipidus of nephrogenic origin proved unresponsive to these agents. The results suggest that in diabetes insipidus chlorpropamide has an ADH-like effect on water balance; the effect seems to be due to a potentiation of ADH rather than to a direct renal mechanism.

The therapy of diabetes insipidus has been oriented toward the oral antidiabetic agents in recent years, since these drugs, particularly chlorpropamide, have been found to moderate the increased water excretion.

Since the first report on the antidiuretic effect of oral antidiabetic agents [2], the subject has extensively been studied. However, neither clinical observations nor animal experiments have been able to throw sufficient light on the pathomechanism involved.

The present investigations have been concerned with the effects of various antihyperglycaemic agents on water balance in diabetes insipidus. The patients studied suffered partly from the hypothalamic, i.e. ADH-responsive, partly from the nephrogenic, i.e. ADH-resistant, type of the disease. The parameters of responses registered in the two groups and their comparative evaluation form the subject of the present report.

Material and methods

Five subjects with vasopressin-responsive, and two with ADH-resistant, diabetes insipidus have been studied. The diagnosis was based on routine endocrine investigations, i.e. daily urinary output, specific gravity of urine, concentration test, oral water and salt loading, Carter—Robbins test, responsiveness to ADH. The patients of the first group had no significant ADH-reserves, nor was a hypofunction of the anterior pituitary lobe or vasopressin-resistance demonstrable. In patients 6 and 7, unresponsiveness to ADH was established on grounds of the intravenous HANKISS-test [10] and of the combined vasopressin-water-loading test [16] as well as by the use of vasopressin tannate of protracted action. The subjects under study were free from liver or heart disease. Their relevant data are summed up in Table I.

The investigations were carried out in six-day periods with the patients on a light mixed diet. During the first period no treatment at all was given. The schedule of dosage of the

Table I

Serial No.	Name	Age (years)	Sex	Relevant data
1	Cs. I.	44	M	Sudden onset of polyuria in the absence of any previous abnormality, 4 or 5 years ago. Daily urinary output 12 to 15 l. Nasal insufflations of Piton R and chlorthiazide treatment.
2	M. J.	51	M	Cranial injury with loss of consciousness in 1941; subsequent onset of polyuria (6 to 8 l daily). No drug treatment.
3	B. S.	32	M	Excessive intake of fluids since 1958. Primary diagnosis, Hand—Schüller—Christian's disease. Rarefactions of cranial bones and maxillae. X-ray and prednisolone therapy. Daily urinary output 13 to 15 l. Nasal insufflations of Piton R and chlorothiazide treatment.
4	S. M.	28	F	In September, 1956, sudden onset of polydipsia and polyuria (9 l daily) after a febrile disease accompanied by diarrhoea and blood in the faeces (diagnosed as dysentery). Therapy: vasopressin tannate in injections, nasal insufflation being poorly tolerated.
5	F. P.	42	M	Cranial injury in 1953, followed by polydipsia and polyuria. Daily urinary output 30 to 40 l. Therapy: vasopressin tannate
6	A. I.	30	M	Polydipsia since early childhood. Pituitary implantation attempted twice in 1956. Daily urinary output 10 to 15 l. No drug treatment.
7	K. I.	17	F	Polydipsia since early childhood. Daily urinary output 25 to 30 l, resistant to treatment.

drugs under study was buformin (Adebit, Chinoin), 50 mg t.i.d.; chlorhexamide (Oradian, Chinoin) 200 mg, t.i.d.; chlorpropamide (Diabinese, Pfizer) 250 mg, t.i.d. Before changing over to the next drug, a drug-free interval was observed until urinary output had returned to the pre-treatment level.

The following parameters were registered daily: urinary output (L/24 hrs), urinary osmolality (mosm/l), serum osmolality (mosm/l), endogenous creatinine clearance (ml/min), osmolar clearance (ml/min), free water clearance (ml/min) and blood sugar (mg per 100 ml).

For the estimation of serum and urinary osmolality, a Thermistor cryoscope of the design of Central Laboratory of Szeged University Medical School was used. Blood sugar was determined by the orthotoluidine method, creatinine by FOLIN and WU's method [8] modified by BROD and SIROTA [5].

Results

Table II sums up the data of the ADH-responsive patients. Buformin and chlorhexamide failed to affect spontaneous urinary output in Case I, while they reduced it to a slight extent in the other patients. The antidiuretic effect of chlorpropamide was significant in four cases, it was only in Case 5 where it failed to exceed 20%. Correspondingly, with the only exception of Case 5, chlorpropamide was the only drug to induce a substantial elevation of urinary osmolality. Serum osmolality, endogenous creatinine clearance and osmolar clearance showed minor variations only. Free water clearance displayed no consistent change in response to buformin or chlorhexamide: while slightly increasing in the first two cases, it moderately diminished in the other three. In contrast, the reduction of free-water clearance induced by chlorpropamide was significant, it approximated zero in Case 2,

Table II

ADH-responsive diabetes insipidus

	Case 1				Case 2			
	Before treatment	Treatment			Before treatment	Treatment		
		buformin	chlorhexamide	chlorpropamide		buformin	chlorhexamide	chlorpropamide
Urinary output, 24 hrs	10.8	10.5	10.5	4.6	7.8	6.9	6.6	2.3
Urinary osmolality, mosm/l	44.7	47.1	55.1	157.4	172.2	173.5	131.8	532.6
Serum osmolality, mosm/l	304.9	289.2	303.9	310.7	292.3	313.4	283.7	309.4
Endogenous creatinine clearance, ml/min	102.9	193.6	184.4	143.4	128.7	160.4	158.3	141.1
Osmolar clearance, ml/min	1.5	2.3	1.2	1.5	2.6	2.4	2.2	1.9
Free water clearance, ml/min	4.5	5.0	6.1	1.7	2.0	2.4	2.3	0.3
Blood sugar, mg/100 ml	88	74	74	79	82	83	81	79

Case 3				Case 4				Case 5			
Before treatment	Treatment			Before treatment	Treatment			Before treatment	Treatment		
	buformin	chlorhexamide	chlorpropamide		buformin	chlorhexamide	chlorpropamide		buformin	chlorhexamide	chlorpropamide
13.1	8.3	7.9	6.4	10.4	8.4	9.6	1.9	35.3	31.2	30.9	27.2
109.4	122.4	105.0	200.4	95.0	108.9	107.3	436.6	57.4	52.2	49.7	43.0
310.2	308.0	322.8	330.9	313.1	313.4	300.4	325.5	308.4	317.4	309.3	306.7
160.2	141.8	129.7	177.6	147.8	140.4	88.3	115.9	170.4	156.9	180.2	192.9
2.5	2.1	2.1	2.8	2.5	2.0	2.2	1.6	4.2	4.1	3.5	3.0
4.4	3.3	4.0	1.7	4.8	3.8	3.8	0.5	20.3	17.4	18.0	15.8
93	79	80	65	82	72	80	67	78	79	71	60

and became negative in Case 4, so that free water absorption ensued. In Case 5 the reduction in free-water clearance was of minor degree. The most distinct fall in blood sugar was induced by chlorpropamide; however, no hypoglycaemic crisis was encountered in any of the cases.

Table III presents the data of the two patients with nephrogenic diabetes insipidus. Here, the agents failed to affect the parameters under study to any significant extent. Even chlorpropamide caused no reduction of urinary output and in Case 7, even its slight increase together with that in free-water clearance was noted.

Since it was chlorpropamide which induced the most marked differences in the two groups, it seemed interesting to compare the data before and after treatment of a representative case of each group.

Fig. 1 presents the data of a patient with ADH-responsive diabetes insipidus (Case 4). The diagram clearly shows that urinary output per minute gradually declined in response to chlorpropamide, amounting to no more than one fifth of the control value between the 3rd and 6th day of treatment.

Table III

ADH-resistant diabetes insipidus

	Case 6				Case 7			
	Before treatment	Treatment			Before treatment	Treatment		
		buformin	chlorhexamide	chlorpropamide		buformin	chlorhexamide	chlorpropamide
Urinary output, 24 hrs	12.1	10.7	12.0	12.1	26.0	26.1	29.6	28.7
Urinary osmolality, mosm/l	77.1	61.9	95.4	93.5	48.4	35.0	29.6	49.8
Serum osmolality, mosm/l ..	301.5	279.8	294.5	320.1	301.3	295.9	271.7	287.8
Endogenous creatinine clearance, ml/min	210.7	189.0	191.2	187.4	227.0	196.1	192.8	204.8
Osmolar clearance, ml/min ..	2.2	1.7	2.7	2.5	3.7	2.1	2.2	3.5
Free water clearance, ml/min	6.2	5.7	5.7	6.3	13.8	16.0	18.3	16.4
Blood sugar, mg/100 ml	84	83	85	76	80	79	83	74

Endogenous creatinine clearance and serum osmolality were not significantly affected, in opposition to urinary osmolality which increased significantly during treatment. Osmolar clearance failed to change significantly in response to chlorpropamide. Free-water clearance diminished distinctly to become negative after the 3rd day.

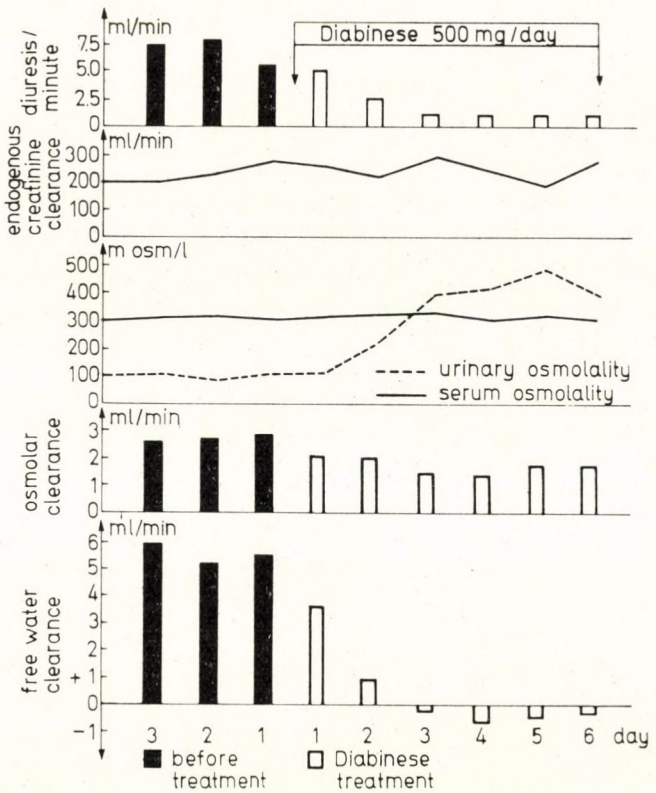

Fig. 1. S. M., 30-year-old female. ADH-responsive diabetes insipidus

Fig. 2 demonstrates the data of a patient with nephrogenic diabetes insipidus (Case 6). It is seen that diuresis was largely unaffected. Urinary osmolality persisted at its former low level throughout the period of treatment and no change occurred in free water clearance, either. The antidiuretic effect characterizing the hypothalamic type of diabetes insipidus failed to appear in its nephrogenic type, in other words, chlorpropamide did not affect the disturbance of water balance in the nephrogenic form of the disease.

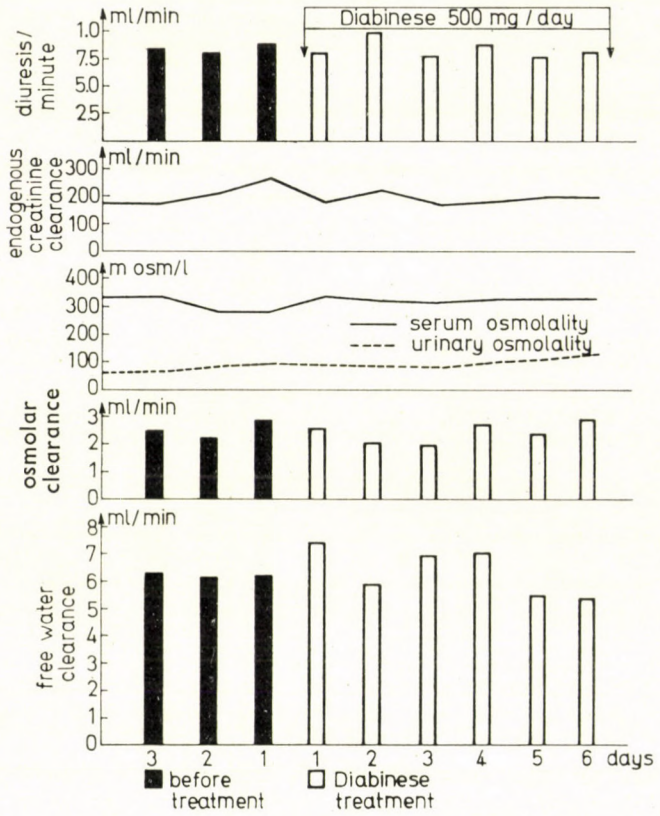

Fig. 2. A. J., 30-year-old male. ADH-resistant diabetes insipidus

Discussion

The effect of the oral antidiabetic agents was studied on water balance in diabetes insipidus. It was chlorpropamide which revealed the most potent antidiuretic activity in ADH-responsive diabetes insipidus, whereas on buformin treatment the fall in urinary output was insignificant. These observations are in line with those of KATSUKI and ITO [13]. On the other hand, REFORZO-MEMBRIVES et al. [26] failed to note any diuretic response to phenformin

in patients with diabetes insipidus. The discrepancy may be accounted for by the dissimilarity of the radical attaching to the biguanide group. Even small modifications of the chemical structure of sulphanylurea derivatives may profoundly affect their antidiuretic activity. Comparison of chlorpropamide and chlorhexamide convincingly illustrates this point. Though there is no other difference between their chemical structures than the presence in chlorhexamide of a cyclohexyl instead of a propyl group, yet the antidiuretic effect of the derivative is far from that of chlorpropamide.

Though the first reports on the antidiuretic effect of oral antidiabetic agents are of recent date, a considerable amount of evidence has been accumulating on the subject, but the pathomechanism still remains uncertain. ARDUINO et al. [2] assume chlorpropamide to stimulate the production and mobilisation of ADH, or to restore the responsiveness of the osmoreceptive centres to physiological stimuli, a hypothesis which, as pointed out by MEINDERS et al. [18] seems to be at variance with the observation that chlorpropamide causes urinary retention in the familial congenital form of diabetes insipidus, the type marked by a total lack of vasopressin. BERGMANN et al. [3] conclude from the results of intraphypothalamic chlorpropamide implantation that the antidiuretic activity of these drugs acts through a prevalently central mechanism, suppression of thirst being its essential factor. However, the view that chlorpropamide acts directly upon the kidney has later gained prevalence [1, 14, 17, 18, 22—27], a reduction in free water clearance having been found in association with an unimpaired excretion of soluble materials (osmolar clearance) in the majority of cases. These observations are in agreement with the results of the present study. On these grounds, chlorpropamide seems to have a direct, ADH-like effect on the kidney, a claim supported by our observation, in agreement with other authors [2, 7, 9, 17, 18, 28] in that chlorpropamide has no antidiuretic effect in vasopressin-resistant diabetes insipidus but reduces urinary output in healthy subjects [20] as well as in patients with psychogenic polydipsia [15].

These facts confront us with the question whether it is actually the same mechanism which underlies the antidiuretic effect of chlorpropamide and of ADH. Evidence available on the subject is insufficient as yet to provide a decisive answer to this question. The results of the classical permeability tests on the frog bladder have also proved inconclusive. While some authors found chlorpropamide similarly to ADH, to enhance the water permeability of the frog bladder [6, 17], others failed to confirm these observations but noted a potentiating effect on the permeability-increasing influence of vasopressin at small concentrations [12, 19]. The results of MILLER et al. [21] and of BERNDT et al. [4] obtained in rats under normal conditions and in congenital hypothalamic diabetes insipidus are consistent with these observations; while chlorpropamide by itself failed to affect polyuria, a striking reduction in urinary

output by small doses of ADH was observed after chlorpropamide pretreatment. On these grounds, chlorpropamide is believed to exert its activity by a potentiation of endogenous vasopressin rather than to have a direct antidiuretic effect of its own.

Certain observations seem to be at variance with the direct renal mechanism of chlorpropamide. In particular, it may fail to reduce urinary output and free water clearance even in ADH-responsive cases of diabetes insipidus [11, 20], as it was in fact observed in Case 5 of the present series. In the cases of MILLER and MOSES [20], chlopropamide antidiuresis was confined to those patients with hypothalamic diabetes insipidus in whom water deprivation also induced a fall in free water clearance. These authors found close correlations between the response of free water clearance to fluid deprivation and to chlorpropamide, and advocate the routine use of the water deprivation test in diabetes insipidus so as to predict the response to chlorpropamide. A further fact pointing to the role of endogenous ADH in the antidiuretic mechanism of chlorpropamide is the observation that loading with ethanol or water which is known to suppress the release of ADH, fully inhibits the chlorpropamide-induced changes of the water balance [20]. These clinical observations lend indirect support to the hypothesis that chlorpropamide antidiuresis takes effect only in the presence of endogenous ADH, the mechanism being attributed to a potentiation by chlorpropamide of the antidiuretic activity of the supposedly very small amounts of vasopressin. The question is, however, by no means closed and awaits support from further studies, in particular from reliable determinations of the ADH- reserve in humans, which would provide an answer to the question whether there existed any positive correlation between the available, i.e. mobilizable, stores of ADH and chlorpropamide antidiuresis.

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ANNOUNCEMENT OF AN IAEA SYMPOSIUM

Title: Nuclear Activation Techniques in the Life Sciences
Date: April 10—14, 1972
Location: Ljubljana, Yugoslavia
Organizers: International Atomic Energy Agency, Kärntnerring 11—3, A—1011 Vienna, Austria
Scientific Secretaries: Dr. G. B. Cook and Dr. R. M. Parr.

The Symposium will be concerned with the applications of nuclear activation techniques in the life sciences and the significance of the results obtained in such applications. It will therefore be concerned both with the techniques themselves and with the interpretation of the data which they yield. A symposium of the same title was held in Amsterdam, Netherlands, in 1967 and this, the second of the series, is intended to cover the advances made in the five-year interval.

As regards techniques, topics to be discussed include sample preparation procedures, activation procedures and data processing systems, chemical separation procedures particularly in multicomponent systems, and biological analytical reference materials. As regards interpretation of data, results obtained in studies in both cellular and subcellular systems in plants and animals will be discussed. Contributions relating to agriculture, biochemistry, ecology, nutritional studies, pharmaceutics and pharmacology, as well as applications in medical diagnosis, research and therapy, will be included. Contributions relating to human ecology will deal especially with problems of public health, environmental pollution and food additives and contamination.

Further information and forms to accompany abstracts of papers intended for presentation at the Symposium may be obtained from national authorities for atomic energy matters. Abstracts must be submitted through these authorities so as to reach the International Atomic Energy Agency before December 13, 1971.

INDEX

ACTA MEDICA

ТОМ 28 — БЫП. 2

РЕЗЮМЕ

НАБЛЮДЕНИЯ В СВЯЗИ С ВОСЬМИ СЛУЧАЯМИ СИНДРОМА БУДД—ХИАРИ

Й. СЁЕР, Д. НАДЬ и С. САКАЛЛ

После краткого изложения синдрома Будд—Хиари авторы сообщают о наблюдении восьми случаев этого заболевания. У четырех из восьми больных причиной болезни была полицитемия, у трех — опухоль печени, у одного облитерирующий гепатический эндофлебит. В трех наблюдениях удалось поставить клинический диагноз. Из восьми больных семь умерли. У одного больного с синдромом Будд—Хиари противополицитемическая терапия привела к ремиссии, которая еще держится. Кроме оценки своих собственных наблюдений авторы дают обозрение о важнейших данных, сообщенных венгерскими авторами в связи с синдромами Будд—Хиари.

ИЗМЕНЕНИЕ СОДЕРЖАНИЯ ТРАНСАМИНАЗЫ В МОЧЕ И СЫВОРОТКЕ КРЫС ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ОТРАВЛЕНИИ ОРГАНИЧЕСКОЙ РТУТЬЮ

К. ЛЕХОЦКИ и Ш. БОРДАШ

Авторы изучали поражающее почечные канальцы действие органического соединения ртути, метокси-этил-меркурихлорида у крыс при хроническом отравлении.

Для изучения затяжного и малого действия, определение активности трансаминазы в моче оказалось очень подходящим. Результат параллельного определения трансаминаз глютамино-щавелеуксусной и пировиноградной кислот в сыворотке доказывает, что повышенная активность трансаминазы в моче происходит из отслоенных и расщепленных эпителиальных клеток канальцев. Это подтверждается многочисленными эпителиальными клетками, наблюдаемыми в осадке. Пробы с концентрацией и разбавлением мочи, исследование белков и сахара не оказались достаточно чувствительными методами для выявления умеренного поражения канальцев.

Метокси-этил-меркури-хлорид вызывает у крыс, помимо известного нарушения функции нервной системы, также и поражение почек, хорошо выявляемое функциональной пробой.

ОБРАЗУЮЩИЙ АКТГ, СЕРОТОНИН И КАТЕХОЛАМИН РАК БРОНХОВ

Л. МОШОНИ, Г. СИЛАДИ, Л. ГРАФ, Е. ПАЛАШТИ и Э. САТЛОЦКИ

Дается описание рака бронхов с множественной секрецией гормонов. Помимо уже известного образования АКТГ и серотонина в опухолевой ткани авторы наблюдали значительное количество катехоламина. До сих пор еще не были опубликованы сообщения о секреции гормона полипептидного характера. Клинические симптомы больного соответствовали чрезмерному образованию катехоламина.

ТОРМОЖЕНИЕ ИММУННОГО ОТБРАСЫВАНИЯ КОЖНОГО ТРАНСПЛАНТАТА ПУТЕМ ПРЕДВАРИТЕЛЬНОГО ЛЕЧЕНИЯ ДОНОРА АНТИЛИМФОЦИТАРНОЙ СЫВОРОТКОЙ. ИММУНОЛОГИЧЕСКАЯ ПОДГОТОВКА ТРАНСПЛАНТАТА

Д. ПЕТРАНЬИ, Д. СЕГЕДИ и Б. ФЕКЕТЕ

Время выживаемости трансплантата мышьей кожи повышается предварительной дачей донору гетерологичной антилимфоцитарной или антитимоцитарной сывороткой. Хорошее действие объясняется, предположительно, кроме торможения донорных лимфоцитов в пересаженной ткани также и образованием антител, защищающих трансплантат.

РЕАКЦИИ ОКОЛОУШНОЙ ЖЕЛЕЗЫ, ВЫЗВАННЫЕ НЕПОСРЕДСТВЕННЫМ РАЗДРАЖЕНИЕМ ПРИ ДЕПРЕССИИ

Я. ЛИПТАК, Д. МОЖИК, П. ВАЦИ и Б. ВАМОШИ

Была изучена секреция околоушной железы до лечения и после выздоровления у 15 контрольных лиц без явлений депрессии (10 мужчин и 5 женщин со средним возрастом в 35 лет) и 20 больных депрессией (14 женщин и 6 мужчин со средним возрастом в 41 год, 1 случай реактивной и 19 случаев эндогенной депрессии). Слюна околоушной железы была получена через механически фиксированную капсулу, а размер секреции характеризована числом каплей в минуту.

При депресси в существенной мере понизилась секреция при покое и также реакция околоушной железы, вызываемая почти всеми способами раздражения (одностороннее механическое и химическое раздражение, вызванное 5 мл 5% лимонной кислотой «симультанная» и «сукцессивная суммация», серийное раздражение). Понижающее секрецию действие эксикоза можно исключить с большой вероятностью. При депрессии выражаемое числом каплей значительное повышение реакции показывает обратную связь с повышением силы раздражения. После излечения начальная низкая секреция достоверно повышается, но не достигает величин, определенных у контролей.

ЗНАЧЕНИЕ ДЛИТЕЛЬНО ДЕЙСТВУЮЩЕГО СТИМУЛЯТОРА ЩИТОВИДНОЙ ЖЕЛЕЗЫ (LATS) И АВТОИММУНИТЕТА ЩИТОВИДНОЙ ЖЕЛЕЗЫ ПРИ ВОЗНИКНОВЕНИИ ЭНДОКРИННОЙ ОФТАЛЬМОПАТИИ С ЕВТИРЕОЗОМ

Я. ФЕЛЬДЕШ, Й. ТАКО, Ч. БАНОШ, и Е. ГЕСТЕШИ

Только у части больных эндокринной офтальмопатией с евтиреозом можно выявить в плазме LATS-IgG. Подобное положение наблюдается также в отношении автоантител против щитовидной железы. Авторы обсуждают значение первичного патогенного значения LATS и автоиммунитета щитовидной железы и рассматривают эти явления скорее как вторичные изменения, хотя в возникновении офтальмопатии может быть речь о их подсобном действии. Наблюдения авторов указывают на то, что различные формы эндокринной офтальмопатии близки друг к другу. Это доказывается тем, что отдельные патологические процессы показывают переходы друг в друга.

АКТИВНОСТЬ РЕНИНА В ВЕНОЗНОЙ КРОВИ ПОЧЕК У СОБАК ПРИ ГИПЕРТОНИИ, ВЫЗВАННОЙ ОДНОСТОРОННИМ ЛИГИРОВАНИЕМ ПОЧЕЧНОЙ АРТЕРИИ

А. ФЕКЕТЕ, И. ФОРГАЧ, К. ГААЛ и Т. МЕСАРОШ

В экспериментах на собаках после полного лигирования почечной артерии одной стороны при невредимой почке другой стороны авторы наблюдали развитие острой или хронической гипертонии. При определении активности ренина в венозной крови ишемической и невредимой почек, а также в периферической артериальной крови было установлено, что ишемическая почка и, после ее удаления, невредимая почка производят прессорное вещество и выделяют его в кровообращение. Развитие гипертонии и поддержание повышенного кровяного давления обуславливаются образованием прессорного вещества ишемической почкой, а после ее удаления невредимой почкой.

ДЕЙСТВИЕ ПЕРОРАЛЬНЫХ АНТИДИАБЕТИЧЕСКИХ СРЕДСТВ НА ДИУРЕЗ ПРИ ЧУВСТВИТЕЛЬНОМ К ВАЗОПРЕССИНУ И НЕФРОГЕННОМ НЕСАХАРНОМ МОЧЕИЗНУРЕНИИ

Ф. А. ЛАСЛО и Л. ЦАКО

Авторы применяли у пяти больных с чувствительным к вазопрессину и у двух больных с нефрогенным несахарным мочеизнурением пероральные противодиабетические средства: 1-бутил-бигуанид (Адебит, Хиноин) и хлор-бензилсулпропамид (Диабинезе, Пфицер), и регистрировали характерные для диуреза данные. Было установлено, что хлорпропамид у четырех лиц с чувствительным к антидиуретическому гормону несахарным мочеизнурением в существенной мере снижает мочеотделение, клиренс свободной годы и повышает осмолярность мочи. Лечение бутил-бигуанидом, а также циклогексилкарбамидом вызывает только умеренные изменения водного обмена веществ. При нефровенном несахарном диабете примененные антигипергликемические средства оказались безэффективными.

Результаты исследований говорят за то, что хлорпропамид обладает также подобным действию антидиуретического гормона влиянием на водный обмен веществ. По мнению авторов непосредственный почечный механизм не является вероятным, и они выдвигают возможность, что хлоропропамид вызывает антидиуретический эффект путем потенцирования действия эндогенного антидиуретического гормона.

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THE THROMBOELASTOGRAPHIC INDEX IN THE PERIODS OF RECURRENCE AND REMISSION OF POLYCYTHAEMIA VERA

By

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(Received February 24, 1971)

The thromboelastographic index, its components and the fibrinolytic activity have been studied in 55 patients with polycythaemia vera. Practically all parameters studied revealed abnormalities during the periods of recurrence and subsided or became completely or partially normalized in the periods of remission. The results are consistent with the prevalence of vascular complications during the periods of recurrence of the disease in contrast to their low incidence during remissions.

Polycythaemia vera, particularly in the absence of treatment or of adequate control, is marked by the prevalence of thromboembolic and haemorrhagic complications [9, 11, 18, 19]. These manifestations are often of fatal outcome or involve irreversible complications [2]. The dual features of enhanced blood clotting and of haemorrhagic diathesis point to the existence of complex coagulation disorders [8, 12]. In the periods of remission ensued by adequate therapy, complications of this kind are rare [5, 10], in other words, haematological normalization goes hand in hand with a fall in their incidence and even with their total absence.

The thromboelastographic procedure described by HARTER [7] measures the elasticity of the blood clot and its changes, thus permitting to follow up the process of coagulation including the conversion of the blood from its fluid state to a gel phase and its subsequent reliquefaction, i.e. fibrinolysis. It also registers the extent of the changes in elasticity and their course *vs.* time. The informative value of the procedure in various diseases of the blood-forming organs as well as in coagulation disorders secondary to other organic diseases is amply documented in the literature [6, 13, 15, 16, 17]. Application of the method and comparative assessment of its results are, however, made difficult by the lack of uniform criteria. In fact, there are diverse, inconsistently used indicators in existence, such as r = reaction time, c = coagulation time, $r + c$ = complete coagulation time, r/c = thromboplastin consumption coefficient, mA = maximum amplitude of the clot, mE = maximum elasticity of the clot, t = specific coagulation coefficient, T = complete coagulation time coefficient, c = biological retraction coefficient, mA/c = thromboelastographic retraction coefficient, etc. In the interest of consistent evaluation of the thromboelastogram, ORLIKOV and STOFER [16] proposed a further coeffi-

cient, the thromboelastographic index (I) which they have derived from a large number of healthy subjects and of patients with various blood coagulation disorders. This index is composed of the three essential and most current parameters of the thromboelastogram, expressing the results with a single figure on the basis of the formula

$$I = \frac{r(\text{min}) \times c(\text{min})}{mA(\text{mm})}$$

According to the literature, the value of the index corresponds in healthy subjects to 1.08 ± 0.98, increasing numerically in the case of hypercoagulability and decreasing in hypocoagulability. Referred to the normal value, it certainly serves as a useful basis for the comparison of the results of individual studies. It must, however, be noted that possible changes of the numerator and of the denominator in the same sense may produce an "overlap" confusing the results. It would therefore seem expedient to give, in addition to the I-index serving for a rapid, reliable comparative evaluation of orientative character, also the r, c and mA values. Fibrinolysis, as an essential parameter of blood coagulation, may be likewise of informative value in blood coagulation defects.

In earlier studies we have demonstrated in the period of recurrence of polycythaemia vera various abnormalities of blood coagulation, subsiding or disappearing altogether in the period of remission [12, 13]. In the present study the thromboelastographic index has been investigated in 55 patients with polycythaemia vera, with special reference to the r, c and mA values as well as to the extent and prevalence of increased fibrinolysis.

Material and methods

The material consisted of 55 patients with polycythaemia vera, 31 males, 24 females, ranging from 21 to 76 (mean 55.6) years of age. Forty patients were in periods of full activity of the process, 15 patients were in remission.

The controls were healthy subjects with normal blood coagulation.

In the periods of activity of the disease, RBC ranged between 5.700,000 and 6.850,000 (mean 6.325,000). All of the patients had some degree of leukocytosis and of thrombocytosis. Splenomegaly was present in 37, hepatomegaly in 32 of the 40 cases. The diagnosis of polycythaemia vera was based upon sternal bone marrow smears and bone marrow biopsy in every case [1]. The bone marrow was hyperplastic and hypercellular, erythropoiesis was in general significantly, myelopoiesis moderately enhanced and there was a marked megakaryocytosis in the majority of cases.

The remission following radiophosphorus or cytostatic treatment was associated with a complete normalization of the blood counts, a reduction of even complete disappearance of the spleno- and hepatomegaly as well as of the subjective symptoms.

The thromboelastographic studies were performed by HARTER's original method [7] by means of a Hellige-thromboelastograph (model 2601D), using fresh whole blood for the tests. The priming time varied between 10 and 30 sec. We measured the reaction and coagulation times (r and c values) in terms of min and sec, the maximum amplitude (mA) in mm, as also the degree and frequency of enhanced fibrinolysis.

Results

In order to obtain normal reference values, the mean r, c, mA and I values were determined in 30 healthy subjects with no coagulation disorder. The following figures were found:

r: 9.19 ± 0.3663
c: 5.25 ± 0.2397
mA: 56.60 ± 1.3558
I: 1.46 ± 0.3360

The parameters thus obtained differed to a certain degree from those given by ORLIKOV and STOFER [16], the normal control values, c, mA and I having been found slightly higher, and r slightly lower, than those noted by the last-named authors.

Results are summed up in Table I. The average values for r, c, mA and I and deviations in the periods of remission and recurrence were grouped separately.

Table I

Group	No.	r	c	mA	I	Enhanced fibrinolysis, per cent
Normal controls	30	8.19 ± 0.3663	5.25 ± 0.2397	56.6 ± 1.3558	1.46 ± 0.336	—
Polycythaemia, recurrence	40	9.83 ± 0.5216	7.9 ± 0.4313	34.75 ± 1.6225	2.15 ± 0.1946	67.5
Polycythaemia, remission	15	10.16 ± 0.8436	7.15 ± 0.4995	50.33 ± 4.2664	1.67 ± 0.3598	46.7

As it can be seen from Table I, the average r and c values were distinctly higher than those of the controls both during recurrence as well as in remission. The differences between the results obtained during remission and recurrence with respect to the r and c values were not significant owing to an overlap of the differences of the averages, in contrast to the mA value which was significantly lower in the recurrence group than either in the control or in the remission group. The differences between the control and remission groups with respect to the mean mA and I values were not significant.

Enhanced fibrinolysis was demonstrable during the periods of recurrence in 67.5% and during those of remission in 46.7%. However, the enhancement of fibrinolysis was in general more marked in the group of recurrence than in that of remission.

None of the patients displayed a normal thromboelastogram during the period of recurrence in contrast to those of the remission group where normal thromboelastographic values were found in 4 (26.6%) of 15 cases.

Discussion

CHIEVITZ and THIEDE [3] in a survey of 250 cases of polycythaemia vera found the direct cause of death to be thrombosis in 100 (40%) and bleeding in 15 (6%) of the cases. Thrombosis occurred in 20 (40%) and bleeding in 15 (30%) of 50 cases of polycythaemia vera reviewed by RIGBY and LEAVELL [18]. CHERBAK [4] reported on a series of 219 patients with polycythaemia vera followed up for more than 10 years. Vascular complications such as thrombosis, thromboembolism or bleedings were found in 139 (63%).

We have been engaged in the study of the clinical, pathological and therapeutic aspects of polycythaemia vera since 1959 and have been able to follow up a total of 111 cases since that time. The occurrence of thrombosis, bleedings or both before admission and before the start of active therapy, was noted in 48 cases, i.e. in 43% of the complete series, in contrast to the group where full remission had been achieved and in which complications of this kind were confined to two cases. In our experience, surgical interventions also involve a higher risk of postoperative vascular complications in the period of recurrence than during remission [14].

According to coagulation studies in polycythaemia vera [2, 8, 9, 12, 13, 15], the prevalent abnormalities are reduced prothrombin activity and consumption, enhanced prothrombin inactivation and fibrinolytic activity, thrombocythaemia greatly exceeding the normal platelet count in the majority of cases, and a thromboelastogram marked by protracted r and c values and a reduction in maximum elasticity (mE).

In the present study the thromboelastographic index was found significantly above its normal value in the period of recurrence. The individual components of I (r, c and mA) were also abnormal. The majority of patients displayed a significantly enhanced fibrinolytic activity during recurrence. Complete or partial normalization of the values was noted in the period of remission. In particular, the thromboelastographic index showed little deviation from the normal control values.

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NEW CYTOSTATIC DRUGS IN THE MANAGEMENT OF POLYCYTHAEMIA VERA

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Dibromomannitol was administered to 26, tetramethyl sulphonyl-mannitol to 34 patients with polycythaemia vera, both drugs in the form of short-term, massive-dose therapy over a period of three years. Full remission was achieved in 23 out of 26 cases of the first and in all cases of the second group. Reversible complications related to the therapy were noted in two cases. The results are confronted with those of ^{32}P and the general lines of cytostatic treatment are discussed.

The demand for new drugs and for other therapeutic weapons suitable for the management of haemoblastoses has its good reasons. In the first place, the individual types of these diseases exhibit fairly different patterns of response to the various drugs and to other therapeutic procedures [2, 3, 12, 29]. This makes it desirable to have a wide range of therapeutic agents at our command so as to adapt them selectively to each particular type. Moreover, the original responsiveness of a process may change in the course of time [28] and require the application of other drugs. The fact that in certain types of haemoblastoses particular drug combinations have been found to give the best results is also of importance. For these reasons, studies and trials of new cytostatic agents and of their combinations are not only justifiable but even amount to a necessity.

Polycythaemia vera occupies a particular place among haemoblastoses, first of all because it may assume a protracted, favourable course as a result of adequate therapy thus giving ample opportunity for the study of its pathological and clinical features. Moreover, polycythaemia vera may be regarded in certain respects as the prototype of myeloproliferative diseases, not only because the proliferative changes associated with the process affect all the three (erythro-, myelo- and thrombopoietic) blood-forming systems but also because numerous cytostatic drugs are efficient in polycythaemia vera, chronic myeloid leukaemia and haemorrhagic thrombocytopenia alike. Therefore, therapeutic observations in polycythaemia are often valid for other myeloproliferative syndromes as well. It was, for instance, the favourable response of polycythaemia vera to 1,2,5,6-tetramethane sulphonyl-D-mannitol (Zitostop®, TMSM in the following) which has made us to use this cytostatic agent in myeloid leukaemia.

Our studies concerned with the clinical, pathological and therapeutic aspects of polycythaemia vera date back to 1959, and thus far we have had 120 patients under treatment and regular follow-up. Our observations relative to ^{32}P therapy have been reported earlier [6, 7, 20, 22]. Since 1967, we have been prevalently using two cytostatic drugs developed in Hungary, dibromomannitol (1,6-dibromol-1,6-D-didesoxymannitol) and TMSM. Our first observations with the drugs have been reported earlier [19, 21]. It seems now of interest to review our observations collected on a large patient material over a period of more than three years and to weigh the advantages of these agents against their possible hazards as also to compare their therapeutic value with that of radiophosphorus.

Material and methods

Dibromomannitol has been administered thus far to 26 and TMSM to 34 patients. Three patients of the first and 10 of the second group had to be given repeated courses of treatment a few months or years later because of recurrences of the process. In this manner 29 courses of dibromomannitol and 44 of TMSM have been given.

The patients were between 18 and 74 years of age (mean, 48.46 years). The criteria of differential diagnosis have been dealt with earlier [17, 18]. At the start of treatment, RBC averaged 6.900,000 (extreme values, 8.200,000 and 5.500,000); WBC, 12,200; platelet count 480,000 in the dibromomannitol group. The respective values were 6.500,000 (extreme values 9.000,000 and 5.600,000), 10,800 and 560,000, in the TMSM group. The majority of the patients had splenomegaly and hepatomegaly. Some kind of therapy had been administered a few months or years earlier to 18 patients of the dibromomannitol group (^{32}P) and to 16 of the TMSM group (^{32}P to 2, dibromomannitol to 4, both to 6).

Both dibromomannitol and TMSM were administered by mouth, the daily dose being 250 mg and 1.0 g, respectively. The average total dose per course of treatment was 6.25 with dibromomannitol and 8.10 g with TMSM. In other words, the dosage scheme was that of short-term, massive-dose therapy (Stosstherapie), no maintenance doses being given.

In cases with excessively high erythrocyte, haemoglobin and haematocrit values or of earlier vascular manifestations (thrombosis, thromboembolic complications, major bleedings), repeated phlebotomies were included in the therapeutic scheme, a total of 1200 to 1600 ml blood having been withdrawn in successive portions.

Results

The criteria of full remission were complete normalization of the blood counts, of the haemoglobin and haematocrit values, disappearance or substantial reduction of spleno- and hepatomegaly, complete relief of all subjective symptoms associated with the disease (headaches, vertigo, impaired concentration capacity, pruritus provoked by bathing, etc.). Apart from 2 cases during 11 years, no vascular complications occurred during the periods of remission. If a single course of treatment failed to achieve full remission, it was repeated with the same cytostatic drug or with ^{32}P 3 or 4 months later.

Remission was invariably heralded by a decline in the leukocyte and platelet counts to be followed by their full normalization 4 or 5 weeks later in the majority of cases. Disappearance of spleno- and hepatomegaly or sub-

stantial shrinking of the organs in size ensued 6 to 8 weeks after completion of therapy. This went hand in hand with a relief of the subjective symptoms. Full normalization of the erythrocyte count, haemoglobin and haematocrit values ensued with both drugs in 8 to 10 weeks after the conclusion of treatment.

Table I sums up the results of cytostatic therapy in polycythaemia including the average doses of dibromomannitol and of TMSM for each course of treatment, the total number of patients in the two groups, the courses of treatment, the number of favourable responses and the duration of remissions in terms of months.

Table I

Drug	Average total dose per treatment, g	Number of patients treated	Number of treatments	Number of responsive cases	Number of complications	Average duration of remission (months)
Dibromomannitol	6.25	26	29	26	3	18.2
TMSM	8.10	34	44	44	—	10.3

The dibromomannitol series comprised 26 patients. Of these, a remission was attained in 23, 3 having responded poorly to the therapy. However, one of the three latter patients died of myocardial infarction two weeks after the start of treatment.

In the two further cases where dibromomannitol failed to give any distinct benefit, ^{32}P was given 4 and 5 months later with success. Complications related to the therapy, i.e. transitory leukopenia and thrombocytopenia responding to transfusions of whole blood and platelets, occurred in two cases, in the first case two, and in the second four weeks after the start of therapy. There was one fatal case, but the cause of death was unrelated to the therapy, the patient having died of multiple microembolisms and of mesenteric vein thrombosis.

The duration of remissions resulting from the first treatment averaged 18.2 months. These values are final, three of the patients concerned having died, and the remaining patients having relapsed in the meantime. Fresh courses of treatment have been made necessary by recurrences in three out of the 23 responsive patients. The second course was successful all throughout; since, however, all three patients are still in remission, no definite figures can as yet be given on its duration.

The TMSM series comprised 34 patients. Full clinical and haematological remission has been achieved in all cases, and still persists in 20. No complication connected with the drug has been noted in any of the cases. At the time being the duration of remission averages 10.3 months. This is, however,

merely a provisional figure which will probably increase in view of the fact that the majority of patients are still in remission.

A second course of TMSM had to be given in view of recurrences in ten cases, in all with success, the patients being still in remission.

Cardiac failure or anginal symptoms, if present, subsided spontaneously or responded more readily to glycosides or to coronary dilators during the periods of remission. All patients still in the age of occupational activity have resumed their former occupation involving heavy physical work in several cases.

Discussion

The prevalence of vascular complications in untreated or poorly controlled cases of polycythaemia vera is very high [2, 5, 9, 11, 12]. CHIEVITZ and THIEDE [1], in a survey of 250 fatal cases of polycythaemia vera found thrombosis to be the direct cause of death in 100 (40%), and major bleedings in 15 (6%). TCHERBAK [27] noted thrombosis, bleedings or both in 139 (63%) of a series of 219 patients with polycythaemia vera. In the material of WATKINS and FAIRLEY [31] of nearly 100 cases the prevalence of vascular complications was as high as 50%. As regards the present series, 48 patients, i.e. 40%, had had vascular complications such as thrombosis, thromboembolism or repeated bleedings before admission, in other words before the start of active therapy, and in 20 cases (16.6%) these complications (cerebrovascular crises, myocardial infarction, etc. had irreversible consequences. The greatly reduced incidence of vascular complications during remission clearly emphasizes the need for treatment even if symptoms of any severity should be still absent.

Radiophosphorus was first applied in polycythaemia by LAWRENCE in 1939 [13] and numerous observations have supported its favourable effect [2, 3, 4, 11, 12, 13, 23, 25, 31]. The main objection against this therapy is based on the highly controversial allegation that it might increase the hazard of leukaemic transformation [16, 24, 30].

As regards the use of busulphan in polycythaemia, opinions are divided [7, 10, 14] in view of the relatively high incidence of leukopenia and thrombocytopenia [5]. However, according to MAURICE and ALBERTO [15] it may be used in well-selected cases under a close control of the blood counts. DEMIDOVA [5] reported on experience with two ethylene imine derivatives, triaziquone and Markofen®; remission was attained in 16 out of 19 cases with the first, and in 18 out of 20 with the second drug. The incidence of leukopenia and thrombocytopenia was fairly high in both groups.

Of the cytostatic agents of Hungarian provenance we have been using dibromomannitol in 26 and TMSM in 34 cases over a period of more than three years. Full remission was achieved in 23 of the 26 patients, i.e. in 88.5%,

of the first group, the duration of remissions averaging 18.2 months. Complications related to the therapy occurred in two cases, and proved responsive to suitable measures. TMSM was given in 34 cases altogether, in all with success. At the present moment, the remissions average 10.5 months in duration, since however the majority of patients are still in remission, its definite duration will be obviously longer. No complications of TMSM treatment have thus far been noted. On these grounds both drugs have been found suitable for the management of polycythaemia vera.

In our experience the question whether the patients have had any other previous therapy does not affect the responsiveness to either dibromomannitol or TMSM.

On confronting the merits of cytostatic drugs with those of ^{32}P, we find on the grounds of many years observations that administration of ^{32}P resulted in full clinical and haematological remission in 95 to 96% and that the remission following the first courses averaged 25.68 months in duration [22]. No complication calling for therapeutic measures has been noted in our ^{32}P-series.

The appearance of acute leukaemia was encountered in one case and that of myelofibrosis in a further case in our ^{32}P-series during the entire observation period of 11 years. The second patient had been given dibromomannitol in addition to ^{32}P. The use of TMSM proved successful all throughout and involved no complications in any of the cases, but the periods of remission are still too short for any comparative evaluation.

Our earlier studies [20, 22] as well as the present observations seem to warrant the use of cytostatic drugs in alternation with ^{32}P. In the early stages, particularly in young patients, cytostatic agents are recommended, since they have been found to bring about adequate remissions in cases of lesser severity, whereas more advanced cases, particularly in the presence of vascular complications or loss of responsiveness to cytostatic agents, call for ^{32}P. A policy of this kind provides for the reduction of the overall doses of ^{32}P which the further course of disease may still require. This is a point of importance since, notwithstanding the occurrence of spontaneous leukaemic transformation even in the absence of any therapy [23, 31], the possibility that this eventuality might be promoted by ^{32}P therapy, cannot be ruled out.

The fact that cytostatic therapy dispenses with the need for isotope laboratories has also its disadvantages since it makes this therapy accessible to those who are not familiar enough with the diagnostic and therapeutic aspects of polycythaemia vera. In reality, the hazards of grave or resistant leukopenia and thrombocytopenia involved by the rapid cytostatic effect call for the closest possible haematological supervision during the entire period of treatment.

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VECTORCARDIOGRAPHIC STUDY OF THE VENTRICULAR SPREAD OF EXCITATION IN PATIENTS WITH ARTIFICIAL PACEMAKER

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Vectorcardiography in patients with artificial pacemaker is suitable for (1) comparative assessment of different vectorcardiographic systems; (2) close study of the stimulus artifact; (3) precise location of the stimulating electrode; (4) electrophysiological analysis of the spread of excitation in human subjects.

Electronic pacemakers are increasingly used in patients with life-threatening conduction disturbances and arrhythmias. The studies concerning the electrocardiographic aspects of pacemakers have been prevalently concerned with the shortcomings of the method and with pacemaker-induced, iatrogenic arrhythmias [11, 12, 15, 16, 21].

The studies on the spread of excitation generated by the pacemaker have greatly contributed to our understanding of electrophysiology. Human pathology involves many phenomena which are not reproducible in the laboratory animal and therefore we have to make use of every method providing closer information on the processes in question without involving major interventions. Vectorcardiography permits a detailed study of ventricular depolarisation and repolarisation [2, 13, 20, 22] from different aspects. We may study in this manner

1) the stimulus artifacts,
2) the QRS-T-loop,
3) the fusion beats.

Material and method

The electro- and vectorcardiograms of 30 patients with pacemakers were studied, right ventricular units having been used in 25 and left ventricular units in 5. Fifteen were of the fixed-rate, 13 of the demand, and 2 of the atrio-synchronized type. ECG and VCG were recorded before and after implantation of the pacemaker or replacement of the battery. The corrected orthogonal Frank lead system was used. The recording apparatus was a Visocard or a Biokomb type vectorcardiograph, the latter of our own modification. Photoregistration of the spikes caused some difficulty, since despite the distinct loops appearing on the screen their direct photographic display was made impossible by the excessive shortness (1 to 2.5 msec) of the sequence of the stimulus spreading. Therefore we had to retouch it in full accordance

with the original. The following parameters were studied in every case: spatial orientation of the stimulus, inscription of the QRS-loop, orientation of the QRS-loop in the frontal, right sagittal and horizontal planes and the precise site of conduction delay.

Results

Stimulus artifact (spike)

The spike of the pacemaker appears on the scalar ECG as a needle-point-shaped potential of extremely short duration. The biphasic nature of the impulse is demonstrable by vectorcardiography (Fig. 1). The initial deflection

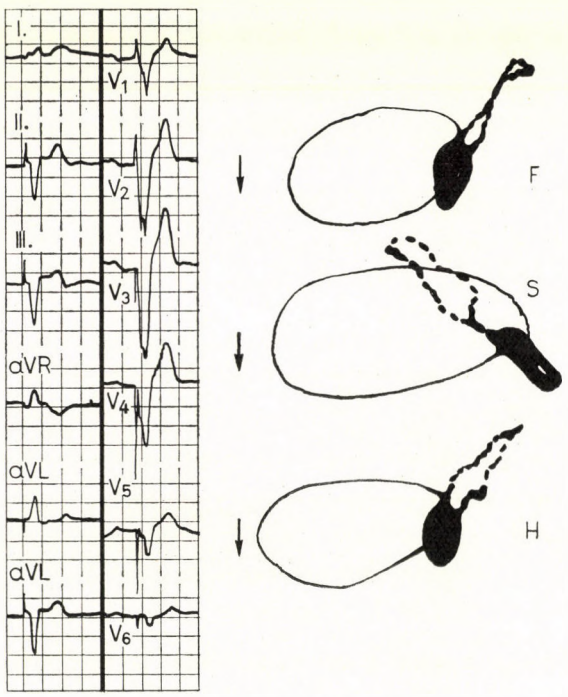

Fig. 1. ECG and VCG of right ventricular stimulation. The continuous loop represents the spike, the interrupted loop the QRS

is a rapid narrow line, subsequently the afferent limb swings suddenly into the opposite direction. This second phase which generally occupies a larger surface area is in fact the result of a condensor-like postdischarge of the tissues.

The amplitude of the spike varies according to the type of pacemaker. The stimulus of a unipolar pacemaker significantly exceeds in height the QRS-loop. This is only natural since it is only the cathode which has an intracardial site the other electrode being placed at some distant point of the body. In the case of a bipolar pacemaker there is no significant difference in

amplitude between QRS and the spike, both poles being placed intracardially approximately 10 mm apart. With a unipolar pacemaker the spatial orientation of the spike depends on the position of the positive pole. The direction varies according to whether the pacemaker is placed in the right or the left pectoral muscle or in the abdominal wall. In the conventional ECG-leads the unipolar pacemaker spike gives the best projection in leads being at right angles to its axis. The direction of the bipolar spike is definable by the line connecting the negative with the positive electrode.

In 11 patients with right ventricular bipolar pacemaker the spike was oriented to the right, anteriorly and superiorly, in 5 patients to the right, anteriorly and inferiorly and in 9 to the left, anteriorly and inferiorly. The great number of cases in which the stimulus was oriented to the left was due in all probability to a reversal of the positive and negative poles. In the case of left ventricular stimulation the spike was invariably oriented to the right, posteriorly and inferiorly. Orientation of unipolar pacemaker stimuli showed no regularity.

Analysis of the QRS-loop

The pacemaker-induced ventricular complexes considerably differ from the supraventricular complexes spreading to the ventricles through normal pathways. It is a simplified empirical rule that pacemaker stimulation of one ventricle produces the ECG-pattern of complete bundle branch block of the opposite side (Fig. 1).

Vectorcardiography provides for a closer analysis of the ventricular spread of stimulus. The pacemaker-generated ventricular complex assumes the pattern of a ventricular premature beat, i. e. of a bundle branch block. The spread of stimulus is likewise similar to that of ectopic beats. The conduction delay is clearly recognizable and precisely localizable in the VCG. The stimulus spreads retrogradely upward from the slow-conducting fibres via the Purkinje network. On comparing the pattern of right ventricular pacemaker stimulation with that of left complete bundle branch block they are found to differ by the location of the conduction delay (Fig. 2). While in complete left bundle branch block it is found in the mid- and terminal portions, with pacemaker stimulation it may be situated in the initial, middle and terminal portions of the QRS.

The sites of conduction delay in the present cases have been summarized in Table I. In both cases, i.e. with pacemaker-generated stimulation as well as in complete left bundle branch block, the stimulus spreads aphysiologically from right to left, with the distinct difference, however, that in the frontal and sagittal planes the complex of left bundle branch block tends to be oriented downward, whereas the pacemaker revolution distinctly points upward. This

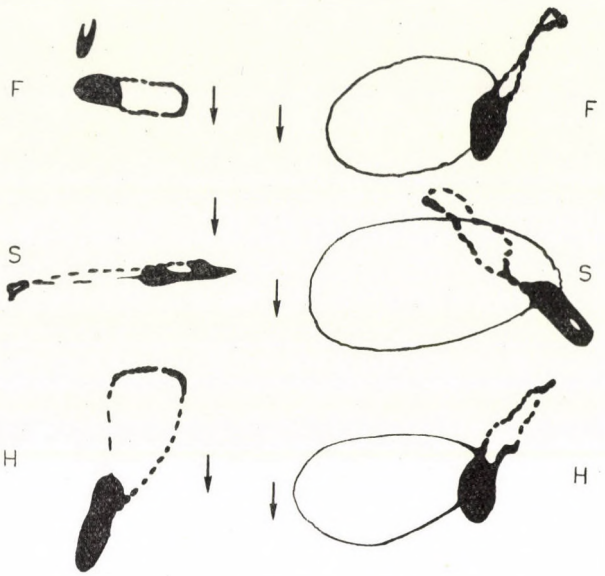

Fig. 2. VCG pattern of right ventricular pacemaker stimulation contrasted with that of complete left bundle branch block

Table I

Location of conduction delay in QRS-loop

Initial and mid-portion	7 cases
Initial, middle and terminal portion	14 cases
Middle and terminal portion	6 cases
Terminal portion	3 cases

shows that in the case of a left bundle branch block there is a special type of septal depolarisation which is not reproducible by stimulation of the right septal surface.

Left ventricular pacemaker implantation results in a pattern similar to that of a right bundle branch block (Fig. 3). The direction of the spread of stimulus depends on the placement of the left ventricular electrode. Posterolateral implantation gives an anterior, while anterolateral implantation a posterior orientation.

Right ventricular pacing may occasionally give rise to an ECG or VCG pattern of the type of complete left bundle branch block (Fig. 4).

In the cases with right ventricular pacemakers the maximal QRS-vector was mostly oriented in the frontal and horizontal planes between 0° and —90°. In 4 patients, despite a right ventricular stimulation, the QRS-loop was oriented in the right lower quadrant. It was assumed that the tip of the elec-

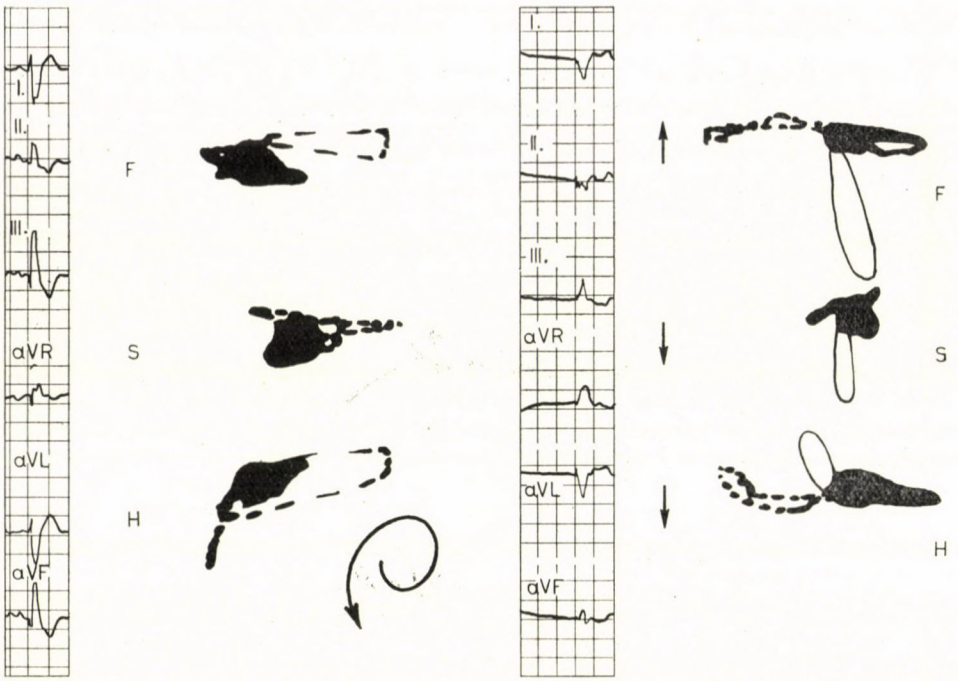

Fig. 3. ECG and VCG patterns of left ventricular pacemaker stimulation contrasted with those of complete right bundle branch block

trode had penetrated the septum. In two patients it was possible in fact to locate the tip of the catheter in the projection of the left ventricle by means of X-rays taken in two views. In the case of left ventricular pacing the maximum QRS vector was oriented in the frontal plane between +120° and —160° and in the horizontal plane between +120° and —150° (Fig. 5).

With *unipolar pacemakers* the stimulating electrode was placed intracardially and the indifferent pole in the pectoral or abdominal musculature. Only a portion of the QRS-loop is recognizable on the VCG, conduction in the striated muscles being unregistrable (Fig. 6).

Demand-type pacemakers provide for successive registration of the pacemaker-generated complexes and of those originating from the sino-auricular node. In the majority of our patients, the pacemaker had been made necessary by AV-block, more precisely by Adams—Stokes attacks associated with coronary heart disease. The onset of complete atrioventricular block had been preceded by intraventricular conduction disturbances (complete left bundle branch block, hemiblock), therefore in patients with demand pacemakers we had the opportunity to compare the pacemaker generated complexes of the bundle branch block type with true bundle branch blocks (Fig. 7).

Identification of myocardial infarction in the case of artificial pacing

Recent or earlier myocardial infarctions are difficult to identify in the ECG or VCG of patients with an artificial pacemaker [3].

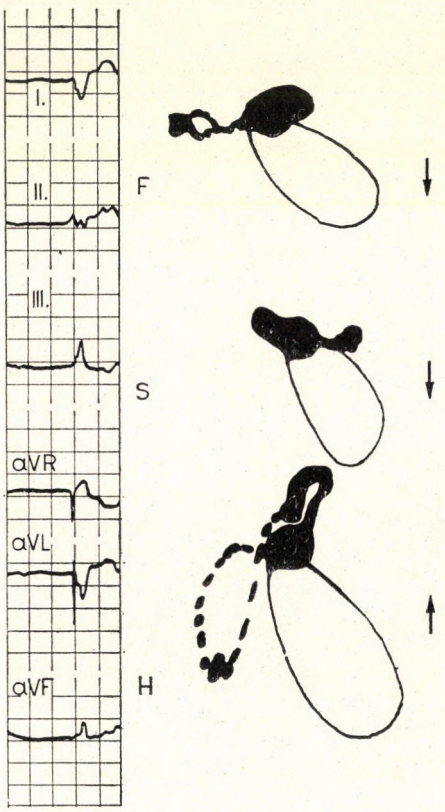

Fig. 4. Right bundle branch block pattern due to perforation of the septum by the electrode

We have been employing the Valsalva-maneuvre for years in order to bring out the signs of myocardial infarction more clearly on the VCG [17]. In one of the patients with a typical history of myocardial infarction, distinct changes in the VCG contours ensued in the course of the Valsalva test (Fig. 8).

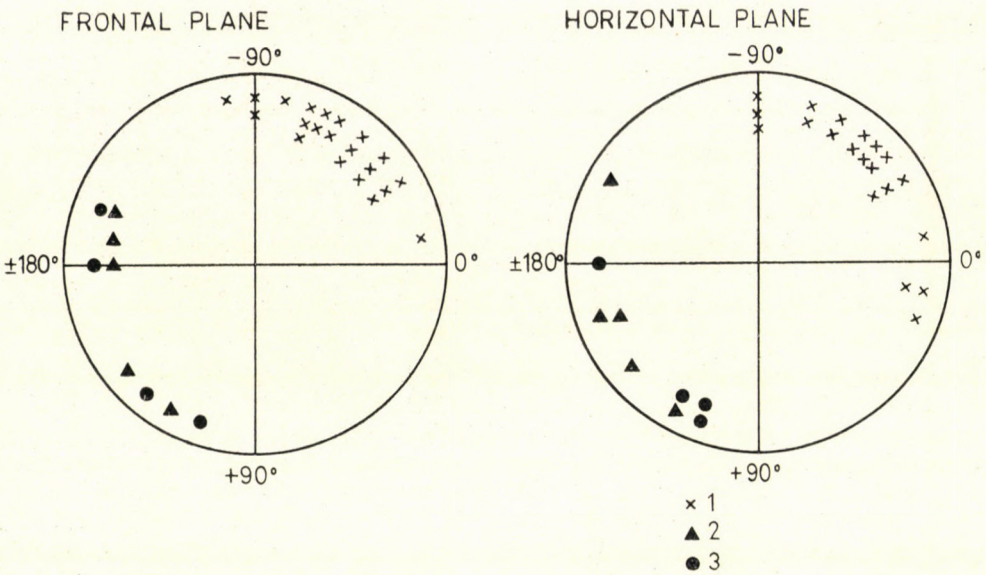

Fig. 5. Orientation of the maximum vector of the QRS-loop in patients with pacemaker. 1. Right ventricular stimulation. 2. Left ventricular stimulation. 3. Right ventricular (atypical) stimulation

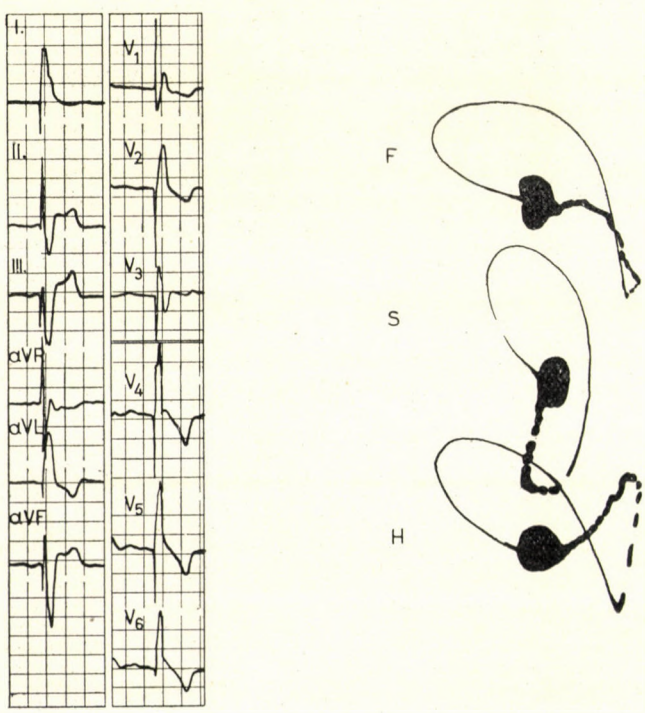

Fig. 6. ECG and VCG patterns of right ventricular unipolar pacemaker

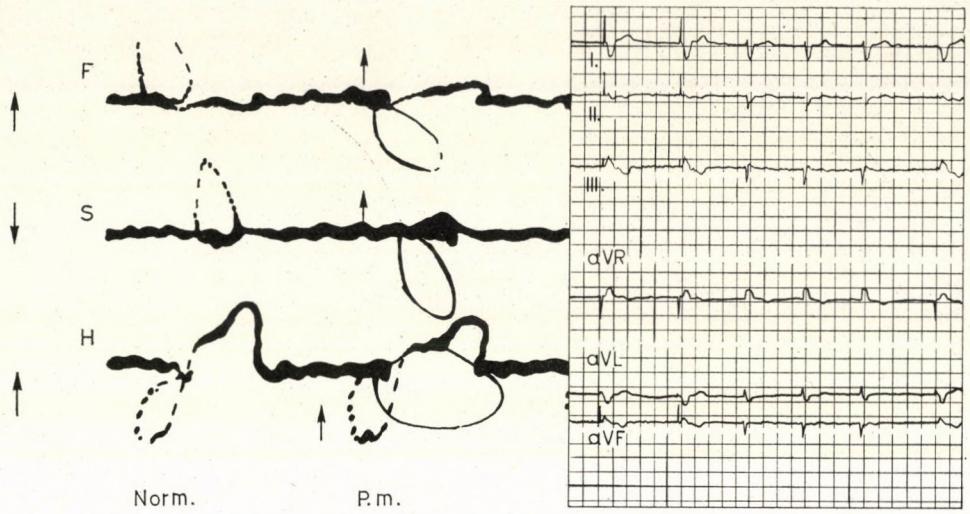

Fig. 7. VCG pattern of true right bundle branch block contrasted with that of artificial right bundle branch block due to left ventricular excitation in a patient with demand-type pacemaker. The difference in the maximum QRS-vector is distinctive of the two types

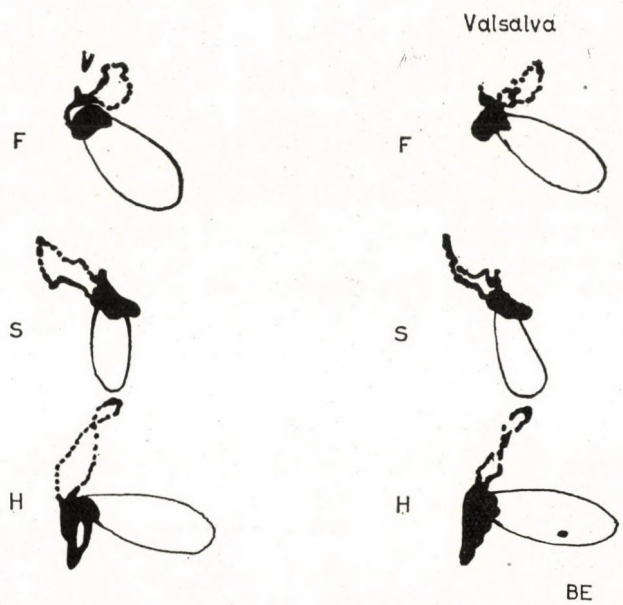

Fig. 8. The Valsalva-test produces a distinct angular distortion of the contour, a sign suggestive of previous myocardial infarction. ↓ = clockwise, ↑ = counterclockwise inscription of QRS

Discussion

On the evidence of the studies of CASTELLANOS et al. [6] the left ventricular bipolar pacemaker stimulus is in 70% of the cases oriented to the right, posteriorly and inferiorly, while the right ventricular stimulus points in 50% to the right, posteriorly and superiorly. GREEN [9] controls the correct functioning of the pacemaker on the basis of the pacemaker vector's orientation. For this purpose he derives the position of the spike from the standard leads in Einthoven's triangle. The frontal pacemaker vector points approximately in the direction of the aVR lead. Reversal of the poles results in a rotation of about 180°. If owing to a displacement of the right ventricular electrode the spike does not point to the apex, the frontal pacemaker vector changes its orientation.

Chest X-rays in two views permit a reliable location of the position of the bipolar electrodes. The spike, as an electric impulse of known magnitude and orientation, lends itself to the standardization of vectorcardiographic systems. ENENKEL [8] used this method for the comparative study of the angular accuracy of the projections of three vectorcardiographic systems so as to find out the margin of error compatible with the projection of an impulse of a given orientation on the individual planes.

BARKER et al. [1] found that excitation of one ventricle results in a QRS-pattern corresponding to that produced by dissection of the opposite bundle branch.

It has been demonstrated by intracardial ECG studies combined with right heart catheterization that the deviation of the QRS axis within the right ventricle varies with the placement of the electrode [5]. At the level of the right ventricular inflow tract in the frontal plane we find a normal R-axis. As the tip of the electrode moves towards the apex, the normal position of the R-axis gradually changes into an extreme left deviation. With the tip at the level of the right ventricular outflow tract the position of the R-axis becomes normal again to assume gradually a vertical position, i.e. an extreme right deviation, as it reaches the subvalvular region of the pulmonary artery. Determination of the position of the R-axis in the frontal plane permits to locate the position of the electrode. An intracardial pacemaker fixed in the apex of the right ventricle involves an extreme left deviation with the R-axis between $-30°$ and $-90°$ in the frontal plane.

The occasional finding of a pattern similar to right bundle branch block in right ventricular pacing is attributed by DANIELSON [7] as well as by STILLMANN [18] to a perforation of the septum or of the anterior wall of the right ventricle by the stimulating electrode, an interpretation which has numerous opponents [4]. Appearance of a right bundle branch block pattern is not necessarily a sign of perforation though in the case of a sudden change

of axis position this possibility should be never left out of consideration. It has been found in the course of cardiac catheterizations that stimulation of the coronary sinus may produce a right bundle branch block pattern [10]. The X-rays permit to locate the pacemaker electrode, in other words to ascertain whether it is situated in the neighbourhood of the septum or of the coronary sinus. MOWER [14] produced experimental evidence of the existence of a circumscribed area of the right ventricular septum, stimulation of which produces the electric pattern of left ventricular stimulation.

SZABÓ et al. [19] thought to define the features of the ventricular complex resulting from pacemaker stimulation by the experimental production of infarction. Differentiation of the ST-elevation and T-inversion thus produced from the repolarisation abnormalities associated with bundle branch block is, however, difficult and even impossible unless a previous ECG is available for comparison.

Pacemaker stimulation is comparable in many respects to premature beat. It is presumed that if the pacemaker stimulation has the neighbourhood of the area of infarction as its origin, it may give rise to ECG and VCG complexes of diagnostic value in the manner of the phenomenon of "infarction premature beat".

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DETERMINATION OF INDIVIDUAL UNCONJUGATED 17-KETOSTEROIDS AND OF 17-KETOSTEROID SULPHATES AND GLUCURONOSIDES IN HUMAN URINE

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A procedure is described for the determination of individual 17-ketosteroids from human urine, excreted as unconjugated metabolites and as sulphate and glucuronoside esters. For the isolation and separation partial hydrolysis, extraction and subsequent paper chromatography, for the quantitative estimation micro Zimmermann-reaction have been used.

In normal individuals, the unconjugated urinary fraction consisted prevalently of etiocholanolone, 11-OH- and 11-keto-etiocholanolone, whereas in the sulphate fraction dehydroepiandrosterone and 5α-steroids, and in the glucuronoside fraction the 5β-reduced 11-oxy and 11-deoxy metabolites predominated.

Changes in the spectrum of the urinary metabolites observed following administration of corticotrophin, Metyrapone, testosterone or dehydroepiandrosterone are also discussed.

The steroid hormone metabolites in urine are mainly sulphate and glucuronoside esters whereas the unconjugated steroids account only for a small fraction. It is suggested that the hormonal and other biological effects are attributed to unconjugated steroids. Sulphate esters are regarded as an inactive reserve of the hormones, and glucuronosides appear to be end-products of steroid metabolism [23, 27]. In the case of enhanced hormone production primarily the excretion of sulphate esters increases, whereas in deficiency syndromes the hormones are metabolized for a greater part to glucuronosides [24]. Recently, in addition to the production, the extent and mode of steroid conjugation have been considered in the assessment of the metabolism and hormone action.

The 17-ketosteroids (17-KS) are products of complex metabolic processes. Dehydroepiandrosterone (D), though having low androgenic activity, is a source of potent androgens and oestrogens and is regarded as a "prehormone" [1]. Its main metabolites, etiocholanolone (E) and androsterone (A) as well as D itself, are excreted in the urine as sulphate and glucuronoside esters and, in a small fraction, as unconjugated steroids. The 17-KS are present in peripheral blood prevalently in the form of sulphates; the renal reabsorption of glucuronosides is insignificant [3]. Further 17-KS, routinely determined in urine, include 11-OH-etiocholanolone (HOE), 11-OH-androsterone (HOA)

and 11-keto-etiocholanolone (OE); they are metabolites of adrenal secretory products.

The importance of a simultaneous estimation of D-sulphate and D-glucuronoside in urine emerges from the observations which demonstrated that the measurement of the cumulative specific activity of these metabolites in the urine after the intravenous administration of [4-^{14}C]D and [7α-^{3}H]D-sulphate allows to study the characteristics of D and D-sulphate dynamics (rates of secretion, production, conversion and of irreversible metabolism).

Based on these observations, a procedure suitable for the separation and quantitative estimation of the individual esterified and unconjugated urinary 17-KS has been elaborated. The method has been derived partly from our procedure described earlier for routine 17-KS fractionation [9] and partly from other methods [5, 6, 10, 26] suitable for a selective cleavage of the individual steroid esters.

The procedure, normal values and results of control experiments and of various function tests form the subject of the present report.

Material and method

Isolation of free steroids

A 50 ml aliquot of 24-hour urine was extracted twice with 50 ml benzene. The combined extract was purified with 20 ml N NaOH and twice with 10 ml water. The aqueous phases were pooled with the remaining urine for further processing. The benzene phase was dried over anhydrous Na_2SO_4 and evaporated to dryness.

Isolation and hydrolysis of sulphates

In an amount corresponding to half volume of the aqueous phase, 20 g $(NH_4)_2SO_4$ was dissolved and the pH adjusted to 1.0 with concentrated H_2SO_4. The steroid conjugates (sulphate + glucuronoside) were extracted twice with 50 ml ethyl acetate and the residual urinary phase was discarded. The pooled extract was incubated at 37 °C for 16 to 24 hours. After solvolysis for cleaving sulphates, the extract was cooled to room temperature, alkalinized with 1 ml concentrated NH_4OH and the glucuronosides were extracted with 20 ml N NaOH and twice with 10 ml water. The residual organic phase was dried over Na_2SO_4 and evaporated. The residue was dissolved in 0.5 ml benzene.

Hydrolysis and isolation of glucuronosides

To the aqueous phase, 10 ml concentrated HCl and 30 ml benzene were added, and the mixture was gently refluxed at 80 to 85 °C for 20 minutes. Then the mixture was shaken, the benzene phase separated and the aqueous phase was repeatedly extracted with 30 ml benzene. The combined extract was washed with 6 ml 10% NaOH, then twice with 5 ml water, dried over Na_2SO_4 and evaporated. The residue was dissolved in 0.5 ml benzene.

Separation and quantitative determination of steroids

The total amount of unconjugated steroids, the sulphates and glucuronosides in an amount of 100 to 200 μg (0.1 to 0.3 ml, depending on the total steroid level), furthermore A serving as reference standard in amounts of 10, 20 and 30 μg and methyl red as marker, were applied on 2×56 cm strips of Whatman No. 1 paper. It was then impregnated with a mixture of propylene glycol–methanol (30 : 70), thereafter equilibrated for 3 hours and chromatographed for 16 to 20 hours in a system of n-heptane–benzene–methanol (130 : 60 : 10).

The chromatogram was developed by Zimmermann reaction according to our method described earlier [10]. The steroid zones were eluted from the paper with 3 ml methanol–water (90 : 10), and evaluated in a Spektromom-360 (MOM, Budapest) spectrophotometer at 520 nm in 1 cm cuvettes using the corresponding calibration curve [10].

Comparative study of glucuronoside hydrolysis

To investigate the efficiency of mild hydrolysis performed under benzene layer, the steroid glucuronosides were submitted to enzymatic hydrolysis [10] and to perchloric acid/ether solvolysis [26]. For this purpose the free steroids and the sulphates were extracted from 100 ml urine samples by the method described above, and the residue was divided into four parts equivalent to 25 ml of urine. One aliquot was submitted to benzene/HCl hydrolysis and the other three were processed as follows.

(i) To a sample adjusted to pH 5.2 with concentrated acetic acid, 1000 U/ml β-glucuronidase (Reanal, Budapest) or ketodase (Gödecke, Berlin) dissolved in 5 ml 1M acetate buffer was added. The system was hydrolysed at 37 °C for 24 hours, cooled, and extracted twice with 50 ml benzene. The combined extract was washed with 20 ml N NaOH and twice with 10 ml water, dried over Na_2SO_4 and evaporated.

(ii) In a sample of the same volume, 20 g $(NH_4)_2SO_4$ was dissolved, it was then adjusted to pH 1.0 with concentrated H_2SO_4 and the glucuronosides were extracted twice with 50 ml ethyl acetate. The combined extract was evaporated to dryness and the residue dissolved in 1% perchloric acid in 50 ml ether. This was incubated at 37 °C for 16 to 24 hours, cooled and washed with 10 ml N NaOH, then with 10 ml water, dried over Na_2SO_4 and evaporated.

(iii) The last aliquot was handled as (ii), except that 50 ml of 3% perchloric acid in ether was used for solvolysis.

Control experiments with labelled dehydroepiandrosterone and dehydroepiandrosterone sulphate

Recovery experiments were performed with labelled D and D-sulphate which, prior to use, had been purified by the following procedure.

Approximately 1 μCi of [4-^{14}C] dehydroepiandrosterone (Amersham), specific activity 57.1 mCi/mM, was chromatographed on Silicagel G layer by a system of benzene–ethanol (96 : 4). Pregnenolone-dinitrophenylhydrazone and cortisol-bis-dinitrophenylhydrazone served as markers. After chromatography, the zone between the two references was removed with an aspirator, eluted with freshly distilled chloroform, evaporated to dryness and dissolved in 10 ml absolute ethanol. The stock solution was checked for activity and stored at —16 °C for not more than one month.

Approximately 5 μCi of [7α-^{3}H]dehydroepiandrosterone sulphate ammonium salt (Philips—Duphar), specific activity 10 Ci/mM, and D-sulphate serving as reference standard were chromatographed as above in chloroform–methanol–NH_4OH (80 : 19.8 : 0.2). After chromatography, the reference substance was developed with concentrated H_2SO_4, the corresponding zone aspirated, eluted with chloroform–methanol (80 : 20), and the eluate evaporated to dryness. Purification of the labelled steroid was repeated by chromatography on Silicagel G layer in ethyl acetate–ethanol–NH_4OH (75 : 25 : 5). The labelled steroid was dissolved in absolute ethanol and stored at —16 °C for not more than two weeks.

To the urine sample 2000 cpm [^{14}C]D or 20,000 cpm [^{3}H]D-sulphate, respectively, was added. After processing of the urine, the labelled steroids were eluted from the chromatogram with ether, the eluates evaporated to dryness and dissolved in 5 ml Liquifluor (NEN Chemicals, USA; 4 g PPO + 50 mg POPOP + 1000 ml distilled toluene). Radioactivity measurements were done on a Packard Tri-Carb Model 3314 liquid scintillation spectrometer operating at a 24% efficiency for ^{3}H and a 55% efficiency for ^{14}C.*

Patient material

The normal values were obtained from inpatients of the Department with no evidence of abnormal steroid metabolism and from healthy persons belonging to the staff. The measurements were made from 24-hour urine samples collected (1) under basal conditions; (2) after the

* Institute of Experimental Medicine, Isotope Laboratory, Budapest.

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Table I

Results of different hydrolytic procedures for the determination of 17-KS glucuronosides

Patient, age, sex	Hydrolytic procedure	17-ketosteroids, mg/24 h					
		HOE	HOA	OE	D	E	A
25 F	β-glucuronidase, 1000 U/ml	0.3	1.0	0.3	—	2.6	2.8
	perchloric acid, 1% ether	—	0.4	0.4	—	0.4	0.7
	HCl, 20% benzene	—	1.8	0.6	—	2.8	5.0
36 F	β-glucuronidase, 1000 U/ml	0.4	0.6	0.5	—	1.0	0.8
	perchloric acid, 1% ether	—	—	—	—	traces	traces
	perchloric acid, 3% ether	0.3	0.3	0.3	—	0.5	0.3
	HCl, 20% benzene	0.3	0.5	0.5	—	2.0	1.5
40 F*	β-glucuronidase, 1000 U/ml	—	0.9	0.3	0.2	8.2	12.3
	perchloric acid, 1% ether	—	0.2	0.1	0.1	0.2	0.3
	HCl, 20% benzene	—	0.6	0.6	0.2	8.3	14.2
32 M	β-glucuronidase, 1000 U/ml	—	0.7	0.2	—	1.2	1.1
	perchloric acid, 1% ether	traces	traces	traces	—	0.2	0.4
	perchloric acid, 3% ether	unmeasurable due to chromogens					
	HCl, 20% benzene	traces	0.2	0.2	—	1.3	2.0
35 M	β-glucuronidase, 1000 U/ml	0.6	0.4	0.8	0.2	3.2	3.0
	Ketodase, 1000 U/ml	0.7	0.4	0.7	0.2	3.4	2.9
	HCl, 20% benzene	0.5	0.3	0.8	0.3	3.5	3.2
42 M	β-glucuronidase, 1000 U/ml	0.5	0.7	1.2	0.1	2.8	2.4
	Ketodase, 1000 U/ml	0.5	0.6	1.0	0.2	2.9	2.0
	HCl, 20% benzene	0.3	0.4	1.0	0.2	3.3	2.5

F = female, M = male, * from urine collected on the day of oral administration of 100 mg dehydroepiandrosterone

intramuscular administration of ACTH (Exacthin, Richter, Budapest), 80 U being injected twice on a day; (3) on the two days of Metyrapone administration in daily doses of 3 g; (4) on the last day of intramuscular administration of testosterone-propionate (Androfort, Richter) in doses of 30 mg daily for five days; (5) on the day and the day after the oral administration of 100 mg dehydroepiandrosterone (Richter).

Results and discussion

Control of the procedure

After extraction of the urinary unconjugated 17-KS with a nonpolar solvent [13], fractional hydrolysis was performed according to BAULIEU and MICHAUD [2]. First the sulphates were solvolysed by a modified procedure of BURSTEIN and LIEBERMAN [5] then the glucuronosides hydrolysed. The critical point of fractional hydrolysis was the splitting of glucuronosides for which the common methods are enzymatic hydrolysis [5, 10], solvolysis with perchloric acid [6, 26] or protected acid hydrolysis under benzene layer [9, 10]. Comparative experiments were performed to control efficiency of the three methods and the data obtained have been summed up in Table I. On the evidence of results, the perchloric acid method has been found inadequate to cleave the glucuronosides. Maximum amounts for E and A have invariably been found with the HCl/benzene method. The D values were identical whether mild acid hydrolysis with HCl or the enzymatic procedure had been used. As demonstrated earlier [10], the protected HCl method involves a slight breakdown of HOE and HOA. However, the chromatographic procedure

Table II

Recovery of dehydroepiandrosterone-4-^{14}C and of dehydroepiandrosterone-7α-^{3}H sulphate from urine

No. of sample	Steroid, cpm added to the sample		Recovery, cpm			Recovery, per cent		
			D	DS	DG	D	DS	DG
1	D-C14	2050	1480	—	—	72.6	—	—
2	,,	,,	1860	—	—	91.2	—	—
3	,,	,,	1650	—	—	81.0	—	—
4	,,	,,	1695	—	—	83.5	—	—
5	DS-H3	23,200	70	20.029	140	0.003	86.5	0.006
6	,,	,,	171	18.700	56	0.008	80.8	0.002
7	,,	,,	22	17.133	237	0.001	73.8	0.01
8	,,	,,	80	24.571	207	0.004	105.2	0.009
9	,,	,,	53	19.549	289	0.002	84.2	0.012
10	,,	,,	83	22.745	257	0.004	98.0	0.011

D dehydroepiandrosterone
DS dehydroepiandrosterone sulphate
DG dehydroepiandrosterone glucuronoside

employed appears to be suitable for the separation and quantitation of the breakdown products, as well. These considerations have made us to select the HCl/benzene procedure for glucuronoside hydrolysis.

For a further control, recovery experiments have been performed by tracer [4-^{14}C]D and [7α-^{3}H]D-sulphate or "cold" E. The results of the isotope study have been summed up in Table II. It can be seen that from the unconjugated fraction [4-^{14}C]D, and from the sulphate fraction [7α-^{3}H]D satisfactory recovery ensured with no mutual contamination of isotopes between the urinary fractions. Extraction of the unconjugated D (72.6 to 91.2%), furthermore solvolysis and extraction of the labelled sulphate ester (73.8 to 105.2%) may be considered of adequate efficiency and selectivity. Moreover, on the evidence of 12 experiments the mean recovery of 10 to 50 μg E was 81.6% (range 63.0—99.0%), which is consistent with results of the isotope study.

As to the sensitivity and specificity of the method, we refer to our earlier paper [9].

Unconjugated 17-ketosteroids in urine

Data concerning the excretion of unconjugated 17-KS by normal males and females have been summed up in Table III, in which only the mean and limits of basal excretion are given. According to the observations, the urine of males contained no free D and that of females only small amounts of the steroid. There was a preponderance of 5β-steroids (HOE, OE and E) in the unconjugated fraction. For instance, excretion of HOA was confined to a single case, and excretion of E was in excess of that of A. As a result, the 5β/5α ratio averaged 3.6 or 6.8, respectively, as opposed to the total steroid values (unconjugated steroids + sulphates + glucuronosides) showing a ratio of about 1.0 [11].

Contrary to the esters forming the prevalent part of 17-KS in urine, free 17-KS were either absent or excreted only in minute amounts. According to our knowledge, the presence of these steroids in urine may have the following causes.

1) A failure of conjugation mechanism in the liver or other tissues known to occur under pathological conditions [14, 19] but to a certain degree also under "normal" conditions.

2) Rehydrolysis of steroids, having been conjugated in the liver or in other organs, may occur *in vivo* prior to their excretion in urine. It is known that certain tissues have sulphatase [28] and the kidney has great glucuronidase activity [25].

3) Since part of the steroids is excreted through the intestine and the intestinal flora contains microorganisms of hydrolase activity [8], the

Table III

Urinary excretion of 17-KS in the form of unconjugated steroids and of sulphates and glucuronosides by healthy subjects

Age, years, sex	Form of excretion	17-ketosteroids						
		HOE	HOA	OE	D	E	A	5β/5α
16—38 F	Unconjugated	0—45+	0—5	0—20	0—22	0—30	0—12	2.1—>10
n = 16		12++	—	6	4	9	5	3.6
20—45	Unconjugated	0—65	—	0—20	—	0—60	0—55	0.8—>10
n = 9		26	—	3	—	22	7	6.8
21—40 F	Sulphate	0—0.2*	0—0.3	0—0.1	0.1—1.6	0—0.5	0.1—0.4	0.1—3.0
n = 9		0.1**	0.1	—	0.8	0.2	0.2	1.1
	Glucuronoside	0.1—0.8	0—0.8	0.2—0.6	0—0.4	1.1—2.9	0.8—3.9	0.8—2.6
		0.3	0.4	0.4	0.1	1.9	1.8	1.4
22—43 M	Sulphate	0—0.2	0—0.6	0—0.1	0.4—3.2	0—0.6	0.4—1.5	0.1—0.9
n = 6		0.1	0.3	—	1.5	0.3	0.8	0.4
	Glucuronoside	0.2—0.6	0.3—1.2	0.3—1.0	0—0.2	1.4—4.0	1.5—4.2	0.8—1.3
		0.4	0.7	0.5	0.1	2.6	2.7	1.0

F = female, M = male, n = number of cases
— not detectable
\+ range
++ mean μg/24 hrs
* range
** mean mg/24 hrs

colon may well represent a site of steroid rehydrolysis. On the grounds of recent evidence [7], certain steroid metabolites transformed by the intestinal microorganisms may appear also in the urine.

According to our observations, the 5β-steroids predominate in the urinary unconjugated fraction. This suggests that the mechanism responsible for the excretion (or formation) of unconjugated 17-KS must be 5β-stereospecific. Certain 5β-steroids, such as E, 5β-pregnanediols are known to have pyrogenic, haemolytic or icterogenic, etc., effect [17], and syndromes with these features may be accompanied by an increase of unconjugated 5β-steroid level in the blood [4, 12]. These aspects may give a significance to the simultaneous study of unconjugated 5β-steroid metabolites in blood and urine under normal and pathological conditions.

17-Ketosteroid esters in urine

Values of the 17-KS sulphate and glucuronoside esters in the urine of normal males and females have been summed up in Table III. Dehydroepiandrosterone, the main metabolite of the sulphate fraction, did not exceed 0.4 mg/24 hours in the glucuronoside fraction. The 17-KS metabolites in urine of females were partly 5β-, partly 5α-steroids, whereas those of males were prevalently 5α-steroids, hence the 5β/5α ratio was found invariably less than the unit in males. Quantitatively, glucuronosides formed the main fraction. In both sexes, the 11-oxygenated metabolites and E were prevalently glucuronosides, the relative amount of A in this fraction being less than that of E. In the age group studied (from 21 to 43 years), no age related difference was demonstrable in the distribution of steroids.

Dehydroepiandrosterone and its sulphate ester are produced mainly in the adrenal. The biological half life of free D does not exceed about 60 minutes, that of D-sulphate amounts to 10 to 15 hours [3, 21, 22]. The D-sulphate by slow hydrolysis is converted *in vivo* into D which is transformed at an excessive rate into various metabolites including E- and A-glucuronosides. Unconjugated D, particularly in the case of high hormone levels, is retransformed into D-sulphate, part of which is directly reduced to E- and A-sulphate in the liver [3, 15]. Formation of the sulphates originating from the endocrine organs, the liver and possibly also from other tissues, is unrelated to the detoxication mechanism, these steroids being convertible into unconjugated steroids of hormonal activity [3]. This is consistent with the results of isotope studies according to which the appearance of sulphates is slow and that of glucuronosides is rapid in urine [3, 22].

Since the unconjugated 17-KS are for the greatest part hormonally inactive breakdown products of 5β-configuration and their urinary excretion

Table IV

Urinary excretion of unconjugated 17-KS and of 17-KS sulphate and glucuronoside esters by healthy subjects before, during and after administration of ACTH, Metyrapone and testosterone propionate

Age, years, sex	Treatment	Form of excretion	17-ketosteroids, mg/24 h						
			HOE	HOA	OE	D	E	A	5β/5α
35 F	—	NC	—	—	—	—	0.03	0.01	3.0
		S	0.2	—	—	0.5	—	0.3	0.7
		G	0.2	0.4	0.3	0.1	1.9	2.0	1.0
	Meto II	NC	—	—	0.02	0.01	0.02	0.01	4.0
		S	—	—	—	1.2	0.4	0.8	0.5
		G	0.1	—	0.3	—	4.0	4.8	0.9
38 F	—	NC	0.01	—	0.01	0.01	0.01	0.01	3.0
		S	—	0.2	—	1.6	—	0.2	< 0.1
		G	0.1	0.2	0.5	0.2	1.4	1.6	1.0
	ACTH	NC	0.07	—	0.3	—	0.05	0.01	>10
		S	0.2	0.5	0.1	3.4	0.4	0.6	0.7
		G	0.3	0.6	1.8	0.2	3.0	2.9	1.5
	Meto I	NC	0.02	—	0.01	—	0.04	0.02	3.5
		S	—	0.1	—	1.9	0.5	0.3	1.3
		G	0.1	—	0.3	0.1	2.4	2.5	1.1
	Meto II	NC	—	—	0.02	0.03	0.08	0.03	3.3
		S	—	—	—	1.7	0.7	0.5	1.4
		G	—	—	0.4	0.1	5.1	2.8	2.0
26 F	—	NC	0.02	—	—	—	0.01	0.01	3.0
		S	—	0.3	—	0.4	0.3	0.2	0.6
		G	0.3	0.8	0.4	0.1	1.2	1.2	1.6
	Testo	NC	0.03	0.01	0.01	0.01	0.03	0.01	3.5
		S	—	0.4	—	0.6	0.4	0.8	0.3
		G	0.2	0.7	0.4	0.2	5.3	3.2	1.6

NC unconjugated
S sulphate
G glucuronoside

F female

Meto I On the 1st day of administration of Metyrapone (3 g)

Meto II On the 2nd day of administration of Metyrapone (3 g)

ACTH On the day after administration of ACTH (80 U twice)

Testo On the last day of testosterone propionate administration (30 mg daily for five days)

Table V

Urinary excretion of unconjugated 17-KS and of 17-KS sulphate and glucuronoside esters by healthy subjects before, during and after administration of dehydroepiandrosterone

Age years, sex	Time of test	Form of excretion	17-ketosteroids, mg/24 h						
			HOE	HOA	OE	D	E	A	5β/5α
35 M	before D	NC	0.03	—	0.01	—	0.06	—	>10
		S	—	0.1	—	3.1	—	0.6	< 0.1
		G	0.3	0.4	0.3	0.2	1.4	2.1	1.0
	during D	NC	0.05	—	0.05	—	0.10	—	>10
		S	—	1.0	—	9.6	—	2.9	< 0.1
		G	0.9	1.0	0.9	1.9	3.8	2.9	1.4
38 M	before D	NC	0.04	—	—	—	—	—	>10
		S	—	0.6	—	0.7	0.5	0.4	0.5
		G	0.3	1.2	0.6	0.2	1.5	1.5	1.1
	during D	NC	0.04	—	—	0.04	0.10	—	>10
		S	0.3	0.3	—	12.3	1.9	1.8	1.0
		G	—	0.6	0.4	0.5	5.2	6.2	0.8
40 M	before D	NC	0.02	—	—	—	—	—	>10
		S	0.2	0.5	—	3.0	—	1.0	0.1
		G	0.4	0.5	0.4	—	3.2	2.1	1.5
	during D	NC	—	—	—	—	0.06	—	>10
		S	0.2	0.7	—	14.8	0.9	2.3	0.4
		G	0.6	0.4	0.4	0.3	8.2	6.5	1.3
	after D	NC	—	—	—	—	0.05	—	>10
		S	0.2	0.4	—	4.0	0.2	1.1	0.3
		G	0.5	0.4	0.5	0·1	4.0	3.1	1.4
21 F	before D	NC	0.01	—	—	—	0.01	—	>10
		S	0.2	0.3	—	0.3	0.3	0.2	1.0
		G	0.2	0.1	0.3	—	1.8	1.6	1.4
	during D	NC	0.02	—	—	0.01	0.03	—	>10
		S	—	0.3	—	6.0	0.4	1.1	0.3
		G	0.2	0.2	0.4	0.4	5.9	4.6	1.3
	after D	NC	0.01	—	—	—	0.01	0.01	2.0
		S	0.1	0.4	—	1.0	0.2	0.3	0.4
		G	0.2	0.3	0.5	0.2	3.5	2.5	1.5

NC unconjugated
S sulphate
G glucuronoside

D dehydroepiandrosterone, 100 mg
F female
M male

is not related to the urinary level of the conjugates, the free fraction seems to be no indicator of hormone production and must be attributed to other metabolic factors. Since, on the other hand, the sulphate esters are transformed into active hormones and the rate of their metabolism is slow, the steroid sulphate excretion may be presumed to be one of the best "urinary indicators" of hormone production, whereas the glucuronoside excretion seems to refer on the activity of the inactivation mechanism.

17-Ketosteroid excretion following ACTH, Metyrapone, testosterone and dehydroepiandrosterone administration

The relevant data are to be seen in Table IV. According to the observations, Metyrapone reduced primarily the amount of 11-oxy-17-KS sulphates and increased the level of all 11-deoxy metabolites. In response to exogenous ACTH, a relative increase in the excretion of sulphates was predominant, and after the administration of testosterone it was only in the amounts of E-glucuronoside, A-sulphate and A-glucuronoside where an increase was noted.

Dehydroepiandrosterone is postulated as a "preandrogen". Its intermediary metabolism has been studied recently by the use of tracers or by the administration of the hormone in pharmacologic amounts. The latter has recently been recommended as a function test [20] with the aim to gain information on the biological role and the ways of transformation of the steroid. The majority of these studies have been based on determinations of D, D-esters and the sulphates and glucuronosides of D metabolites [3, 15, 22, 27].

The excretion of individual 17-KS metabolites following administration of D is presented in Table V. As it can be seen, D-sulphate represents the chief metabolite of exogenous hormone, the other metabolites comprise, in the order of magnitude of their urinary level, E- and A-glucuronoside, and E- and A-sulphate. Under physiological conditions, 10 to 20% of the exogenous D appeared in the urine in the form of these metabolites, for the greater part on the day of intake. The amounts of unconjugated 17-KS and of the 11-oxygenated 17-KS esters were scarcely affected by exogenous D.

Our findings on exogenous D metabolism are in agreement with the data of other authors [3, 18]. Observations performed by the present method regarding D and D-sulphate metabolism as well as changes in the 17-KS excretion in the form of sulphate and glucuronoside esters under pathological conditions will form the subject of a further report.

*

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TONOGRAPHIC STUDIES IN FUCHS'S COMBINED CORNEAL DYSTROPHY

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The tonographically determined rate of aqueous flow in 17 eyes affected by Fuchs's combined corneal dystrophy was found to be an average of 0.49 ± 0.37 cu. mm/min. This value is to be considered as decreased in spite of the errors of the method. Decreased formation of the aqueous humour may indicate nutritional disturbances to the cornea and it seems probable that the alteration of the posterior barrier (cornea guttata) and the decreased formation of aqueous humour are complementary conditions of the development of Fuchs's combined corneal dystrophy.

FUCHS [5] was the first to describe in 1910 the combined corneal dystrophy which was to be named after him under the term Dystrophia epithelialis corneae. The primary nature of the endothelial abnormalities was first pointed out by KRAUPA [7]. The observations of this author were borne out by later evidence (DUKE-ELDER and LEIGH [4]). The aetiology of the process is, however, still obscure.

The tonographic studies which form the subject of the present report have been undertaken for the measurement of the rate of aqueous flow in Fuchs's dystrophy.

Material and method

Nine patients with Fuchs's dystrophy were studied. The process was bilateral in all cases but one. The rate of aqueous flow was calculated on the basis of the equation $F = (P_0 - P_v) \cdot C$. P_0 was determined by differential tonometry using a Schiötz tonometer, on the basis of the 1955 nomogram of Friedenwald. In the majority of cases applanation tonometry was not practicable on account of the unevenness of the surface. The venous pressure was considered to be 10 mm Hg. The C value was determined by means of 4-minute tonography.

The studies were invariably performed in the morning hours.

Results

The results obtained in 17 eyes with Fuchs's dystrophy have been summed up in Table I.

Of the average values studied, those of F were distinctly below the normal level. The differences between the values found in early or advanced stages of the process were slight. The mean value of F was 0.58 ± 0.40

Table I

No.	Age	Sex	P_0 (mm Hg)	C	F (cu.mm/min)	Stage
1	65	♀	11	0.11	0.11	Advanced
			11	0.25	0.25	Early
2	70	♀	11	0.34	0.34	Advanced
3	64	♂	16	0.11	0.66	Advanced
			16	0.18	1.08	Early
4	59	♀	23	0.03	0.39	Advanced
			19	0.09	0.81	Early
5	78	♀	12	0.15	0.30	Advanced
			12	0.15	0.30	Advanced
6	62	♂	13	0.19	0.57	Advanced
			11	0.07	0.07	Advanced
7	45	♂	17	0.17	1.19	Advanced
			17	0.17	1.19	Advanced
8	64	♀	11	0.11	0.11	Advanced
			11	0.19	0.19	Early
9	75	♀	12	0.15	0.30	Advanced
			13	0.15	0.45	Advanced
Mean $\pm$ SD			13.9 ± 3.5	0.15 ± 0.09	0.49 ± 0.37	

cu.mm/min in the four early and 0.46 ± 0.39 cu.mm/min in the 13 advanced cases, the difference being not significant ($p < 0.60$). The process was considered to be in the early stage if a distinct cornea guttata was already associated with slight central oedema of the corneal stroma and the other eye revealed marked signs of Fuchs's dystrophy.

Table II

Age	Sex	Side	P_0 (mm Hg)	C	F (cu.mm/min)
70	♀	Affected (Fuchs's dystrophy)	11	0.34	0.34
		Unaffected	15	0.34	1.70

In Table II, the data for the affected and the unaffected eye of patient No. 2 have been set out separately. It can be seen that the rate of aqueous flow was definitely reduced in the affected eye.

Discussion

BECKER, KESKEY and CHRISTENSEN [1] estimate the normal average rate of aqueous flow at 2.3 cu.mm/min. In the view of DUKE-ELDER and GLOSTER [3] the normal rate of aqueous formation, taking into consideration the findings of the various authors and the sources of error of the methods, ranges between 1 and 5 cu.mm/min. The present study yielded a mean of 0.49 ± 0.37 cu.mm/min in the eyes with Fuchs's dystrophy, which value may be considered as definitely decreased in spite of the errors of the tonographic method.

On these grounds we feel justified in connecting Fuchs's dystrophy with a decreased formation of aqueous humour. Since the entire supply of glucose to the cornea and the oxygen supply to the corneal endothelium come from the aqueous humour, an impairment of aqueous formation may interfere with the adequate nutrition of the cornea, particularly in advanced age when the glucose concentration of the aqueous humour declines (POHJOLA [10]). This is consistent with the observation that cataract extraction or cyclitis may adversely affect the process of Fuchs's dystrophy. Thus GOLDMANN [6], MILLER, KESKEY and BECKER [9] and LEE and TROTTER [8] noted that cataract extraction or cyclodialysis was generally followed by a prolonged hyposecretion of aqueous humour and this is very often the case after cyclitis too. In the 17 eyes with Fuchs's dystrophy examined in the present study, the deterioration of the process had been preceded by cataract extraction in 5, by iridencleisis in 1, and by iridocyclitis in 2 cases.

Six eyes of the present series exhibited a C-value below 0.15. According to BECKER and SHAFFER [2] decreased formation of aqueous humour may affect trabecular function.

Cornea guttata, the primary endothelial lesion in Fuchs's dystrophy, represents in itself mainly an involutionary process associated with ageing which need not develop into Fuchs's dystrophy in every case. This would suggest that the conditions of Fuchs's dystrophy are both the abnormalities of the posterior barrier and the deficiency of nutrition caused by the decreased formation of aqueous humour.

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THE TOTAL INTRAPANCREATIC PLASMA PROTEIN SPACE IN RATS UNDER NORMAL CONDITIONS AND IN ACUTE PANCREATITIS

EFFECT OF THORACIC DUCT LIGATION ON THE MORTALITY IN ACUTE PANCREATITIS

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The total intrapancreatic plasma protein space was studied by means of ^{51}Cr protein and ^{131}I albumin in Wistar and CFE albino rats, and 3 hrs after intraductal injection of trypsin and saline. Intraductal injection was also combined with thoracic duct ligation and the dry residue of the pancreas was estimated. The mortality rate was recorded after 24 hrs. The total plasma protein space of the intact pancreas was found to correspond to 23 ml/100 g wet pancreas tissue; this value was increased two-fold by intraductal trypsin. The dry residue amounting to 27% in the untreated pancreas was as low as 16% in the trypsin-treated organ. While in the saline-treated pancreas 3 hrs after thoracic duct ligation a significant expansion of the total intrapancreatic plasma protein space was demonstrable, the mortality of the animals with trypsin-induced pancreatitis showed a three to fourfold increase.

In acute pancreatitis vasoactive substances may be formed in the pancreas [1, 2, 3] which are known to induce local vasodilatation [4—7] and thus increase the amount of blood flowing through the pancreas [6]. They induce histamine-type injury of the vascular endothelium resulting in an increased permeability of the capillaries, venules and even of the arterioles [9]. Consequently, the plasma proteins gain access in excessive amounts to the interstitial space and oedema of the pancreas develops. From the plasma proteins invading the interstitium vasoactive substances are formed [10] which further aggravate the inflammatory process. The progressively growing oedema may compress the pancreatic vessels [11]. Impairment of local blood supply involves further damage to the organ.

From the mechanism outlined above it follows that acute pancreatitis must be accompanied by an expansion of the total intrapancreatic plasma protein space. It seemed interesting to study this question in view of the lack of knowledge concerning the volume of the total intrapancreatic plasma protein space in normal conditions and in acute pancreatitis, and such data could furnish information on the permeability of the vascular endothelium in the pancreas [12—15].

Local lymph circulation is an essential factor in the production of regional oedema [16] as well as in the transport of proteins having gained access to

the interstitial space [17]. In addition to proteins, enzymes and even substances capable of initiating inflammation are transported from the pancreas by the lymphatics [18]. This raises the question whether the thoracic duct ligation affects the expansion of the total intrapancreatic protein space and increases the mortality in acute pancreatitis.

Material and method

Home-bred Wistar or CFE albino rats were used. The animals were grouped at random care being taken to have an equal number of animals from each group for each experiment. Acute pancreatitis was induced by intraductally injected 5 mg trypsin in 0.2 ml physiological saline [19]. The animals serving as controls were subjected to intraductal injection of 0.2 ml physiological saline or to laparotomy only. Each experimental group was divided into two parts. In one part the thoracic duct was left unaffected, in the second its abdominal portion was ligated [20] in order to block the lymphatic drainage from the pancreas.

A total of 329 rats of 250 to 300 g body weight, under intraperitoneal sodium pentobarbital anaesthesia (4 mg/100 g body weight) was used in the present experiments, and we studied the total intrapancreatic plasma protein space 3 hrs after the retrograde injection, as well as the 24-hour survival of the animals.

1) The total intrapancreatic plasma protein space [12, 14] was estimated in 69 animals by means of ^{51}Cr protein prepared [21] in the Isotope Laboratory of our Institute, giving 0.4 μCi/rat of $^{51}CrCl_3$ of 26.6 Ci/g or 74.0 Ci/g specific activity. The intrapancreatic albumin space was estimated in 23 animals by means of ^{131}I albumin supplied by the Hungarian Isotope Institute (1 μCi/rat; specific activity 0.025 mCi/mg). The labelled protein or albumin was injected into the inferior vena cava simultaneously with the intraductal saline or trypsin injection or with laparotomy. Three hours after the intraductal trypsin the animals were killed by bleeding from the abdominal aorta. The pancreas was removed, weighed, homogenized in 5 n NaOH and its activity was measured in a Siemens well-type scintillation detector, activity of the labelled protein being determined in 1 ml blood plasma. In the pancreas homogenate and in the plasma of a few animals, the protein-bound radioactivity was estimated after precipitation with 10% trichloroacetic acid, the residue was taken up in 5% NaOH. In the ^{51}Cr protein assays, in 1 ml plasma 70 to 90% of the activity was protein-bound; in 1 g pancreas tissue, 70%; and in the ^{131}I-albumin assays, in 1ml blood plasma 90% was bound to protein.

To determine the dry residue of the pancreas, the organ samples were dried to constant weight and homogenized in 5 n NaOH. Radioactivity of 1 g tissue was expressed in percentage of the radioactivity of 1 ml plasma. The total intrapancreatic plasma protein space was given in terms of ml/100 g wet pancreas.

The total intrapancreatic plasma protein spaces of the trypsin-treated and the solvent-treated pancreas were compared after analysis of variance by the method of orthogonal contrasts. For statistical evaluation of the dry residue of 100 g pancreas tissue, analysis of variance and subsequently SCHEFFÉ's test was used.

2) Survival was recorded in 237 animals 24 hrs after the intraductal injection. The mortality was evaluated by the χ^2-test and by FISCHER's hypergeometric test. The efficiency of the treatment was checked at autopsy. While in the trypsin-treated animals pancreatic oedema and haemorrhages, in many cases with ascites, were found, the changes in the saline-treated animals were confined to a slight local oedema and in those subjected to thoracic duct ligation only, to chymus extravasates involving the mesenterial root, the posterior abdominal wall and the pancreas. Combination of treatment with thoracic duct ligation was found to enhance the extent and intensity of the lesions described above, particularly in the trypsin-treated pancreas.

Results

1) The values for the total plasma protein and albumin spaces of the intact and trypsin-treated pancreas are shown in Table I. The 3-hour total plasma protein or albumin space of the intact pancreas corresponded to 23 ml/100 g

Table I

Intrapancreatic ^{51}Cr protein and ^{131}I albumin space 3 hours after intraductal trypsin injection

Group	ml/100 g wet pancreas					ml/100 g pancreas dry residue	
	^{51}Cr protein space		^{131}I albumin space			^{51}Cr protein	
	n	Total	n	Total	Protein-bound	n	Total
Trypsin + thoracic duct ligation	14	$34.6 \pm 3.9^{*a}$	4	45.5 ± 7.3^{b}	40.0 ± 7.2^{b}	14	194 ± 26^{a}
Trypsin	14	$48.8 \pm 3.9^{c,a}$	5	44.6 ± 1.5^{b}	39.7 ± 1.5^{b}	14	307 ± 31^{aa}
Physiological saline + thoracic duct ligation	9	36.8 ± 1.6^{a}	5	32.8 ± 4.5	30.4 ± 4.2^{c}	9	195 ± 9^{a}
Physiological saline	7	19.5 ± 1.6	—	—	—	7	93 ± 10
Thoracic duct ligation	12	23.2 ± 1.5	5	25.4 ± 1.5	23.4 ± 1.5	12	94 ± 7
Intact	13	22.7 ± 7.3	4	22.9 ± 11.5	20.0 ± 10.08	13	92 ± 33

$^{a} = p < 0.001$ $^{b} = p < 0.01$ $^{c} = p < 0.05$ n = number of rats
* = mean $\pm$ S. E.

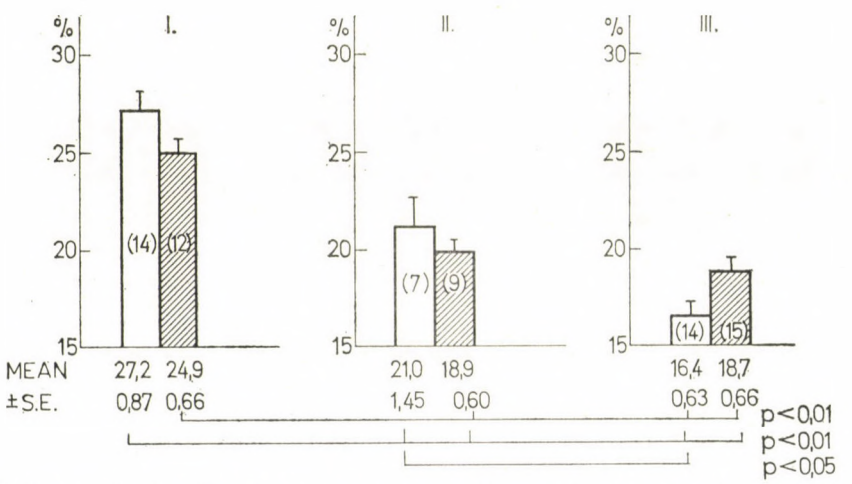

Fig. 1. Dry residue per 100 g wet pancreas tissue 3 hours after intraductal trypsin injection. I. Intact pancreas. II. Pancreas after intraductal injection of 0.2 ml saline. III. Pancreas after intraductal injection of 5 mg trypsin in 0.2 ml saline.
□ No thoracic duct ligation, ▒ thoracic duct ligation, () number of rats

wet pancreas. The highest value was observed after intraductal trypsin injection. Thoracic duct ligation induced no further increase in it. Combination of intraductal injection of physiological saline with thoracic duct ligation resulted in a distinct increase in total intrapancreatic plasma protein and albumin spaces, though to a significantly slighter extent than

after intraductal trypsin. Neither intraductal saline, nor thoracic duct ligation by itself increased the total intrapancreatic plasma protein space. The intact pancreas was found to contain 27% and the saline-treated one 21% dry residue ($p < 0.05$) (Fig. 1). The most extensive pancreatic oedema was thus produced by intraductal trypsin (Fig. 1). The thoracic duct ligation caused no oedema and did not increase the trypsin- or saline-induced oedema (Fig. 1).

2) The thoracic duct ligation resulted in a significant increase in the 24-hour mortality in trypsin-induced pancreatitis (Table II).

Table II

Mortality of rats 24 hours after intraductal trypsin injection

Treatment	Trypsin	Trypsin + thoracic duct ligation	Physiological saline	Physiological saline + thoracic duct ligation	Ligation of thoracic duct	Laparotomy
Number of rats	45	47	20	51	37	37
Died	7	26*	—	2	2	2

$p^* < 0.01$

Discussion

Total equilibration of labelled protein or albumin with the complete plasma protein or albumin pool in rats takes place within three hours [22]. The numerical values for the total plasma protein space of the intact pancreas are in conformity with those of the 24-hour labelled intraorganic protein space of the kidney or the lung [14]. The large total plasma protein space of the kidney may be attributed to the practically free permeability to protein of the medullary vessels [23]. On the evidence of ultramicroscopical studies the permeability of the capillary endothelium to large molecules is slighter in the pancreatic than in the renal vessels [24].

It has been shown earlier that in acute pancreatitis the local oedema is extracellular as well as intracellular [25]. In the present study pancreatic oedema was quantitatively determined from the dry residue the reduction of which as a result of oedema has been found to correlate well with the expansion of the total intrapancreatic plasma protein space.

However, the question why thoracic duct ligation failed to increase the total intraorganic protein space of the trypsin-treated, and the water content of saline-treated pancreas, has yet to be answered. It may be alleged as an explanation that the 3-hour interval may have been too short for the thoracic duct ligation to take effect.

The mechanism accounting for the expansion of the intrapancreatic plasma protein space seems to have been clarified by the present observations.

In view of the excessive leakage of methylene blue into the trypsin-treated dog pancreas, acute pancreatitis induces an increase in local vascular permeability [26]. We have shown earlier that in bile salt-induced acute pancreatitis there is a direct injury to the vascular endothelium, and in sunflower oil-induced pancreatitis, a separation of the junctions between adjacent endothelial cells accounts for the increased vascular permeability [9]. Vascular leakage of the histamine type [8] is suggestive of a local release of vasoactive products in pancreatitis [1, 2]. Vasoactive substances induce, in addition to vascular leakage an increase in pancreatic blood flow. In earlier studies we have shown that histamine, serotonin or kinins injected into the arteries of the pancreas increase the amount of pancreatic blood flow [27]; in other studies we have demonstrated that bile and even saline injected into the pancreatic duct have a similar effect [6]. The later stage of acute oedematous pancreatitis is also associated with an increased local blood flow [28], and any increase in local blood flow favours the passage of plasma proteins across the vascular walls of increased permeability [12, 13].

The question why thoracic duct ligation increases the mortality of rats with trypsin-induced pancreatitis is still unclear. Our earlier studies seemed to suggest that the lymphatics of the pancreas fulfill the function of a safety valve, the pancreatic lesions being aggravated in dogs by ligation of the thoracic duct [29]. In acute pancreatitis the enzyme content of the pancreatico-duodenal lymph is higher than in the blood of the pancreatico-duodenal vein [30], and other authors have shown substances produced by retention of excess pancreatic secretion and capable of initiating inflammation, in the lymph of thoracic duct [18].

We have to consider the possibility that intrapancreatic application of active trypsin may promote the local formation of kinins [31]. Moreover, the thoracic duct ligation may lead to an enhanced production of vasoactive substances from the proteins retained in the pancreas and in the ascitic fluid, in view of the fact that the thoracic duct ligation may promote the retention of proteins [10] in the pancreas by interfering with their transport by the local lymphatic pathways.

*

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PRODUCTION OF AUTOLOGOUS IMMUNE COMPLEX GLOMERULONEPHRITIS IN RATS BY INJECTIONS OF HETEROLOGOUS RENAL TUBULAR ANTIGEN

By

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Autologous immune complex (AIC) glomerulonephritis has been induced in rats by repeated intraperitoneal injections of human renal tubular antigen. The developing renal lesion resulted in proteinuria and a kidney disease similar to that in the experimentally induced (Heymann type) autoimmune nephrosis in the rat and to human membranous nephropathy. A hypothesis is presented which might explain the development of this progressive renal disease under the present experimental conditions.

Introduction

The production of glomerular lesions in rats by the repeated intraperitoneal injections of autologous, isologous, homologous and heterologous renal tubular antigens is well documented [1—5].

The amount of kidney antigen needed to initiate the disease is small. Four injections of 2 mg mitochondrial preparation or a single injection of 3 μg purified antigen is sufficient [6, 7]. Since the disease is progressive it is thought that autologous antigens must contribute to the formation of immune complexes in the glomeruli.

It was previously believed that the disease was initiated and maintained by lymphoid cells since it appeared to be transferable to suitable recipients by lymphoid cells obtained from rats with AIC glomerulonephritis [8, 9]. However, these early claims have not been substantiated [10—12]. Presently it is believed that the renal lesion is produced initially by the injected antigen and antibody formed against it [4] and in the chronic phase maintained by immune-complexes made up of autologous renal antigen, autologous immunoglobulins and complement [3, 13].

EDGINGTON, GLASSOCK and DIXON [14] and HEYMANN et al. [5] have shown that not only autologous, isologous and homologous kidney preparations but heterologous (human, rabbit and guinea-pig) renal antigens can also produce a morphologically similar renal disease in rats.

Since membranous nephropathy in the human resembles morphologically and functionally the experimentally induced AIC glomerulonephritis in the rat, a considerable amount of work has been done to discover the immuno-

pathological processes which are involved in the initiation and progression of this disease.

In previous experiments we have tried to elucidate those immunological events which contribute to the pathogenesis of this experimentally induced autoimmune kidney disease. The cellular immune system appears to play no direct role in the maintenance of the condition [6, 10—12]. On the other hand circulating and glomerular fixed anti-kidney tubular antibodies and a renal tubular antigen appear to contribute to the pathogenic mechanism [3, 15].

In the present experiment we induced AIC glomerulonephritis in rats by repeated intraperitoneal injections of human azo-kidney mitochondrial antigen in complete Freund's adjuvant. An attempt is made to explain those processes which lead to and result in this type of chronic progressive kidney disease.

Materials and methods

Preparation of renal antigen

A fresh normal human kidney was obtained from a young donor who died in a road accident. Mitochondrial preparation (F_3) was obtained by differential centrifugation using the method of PINCKARD and WEIR [16]. The mitochondrial fraction was chemically modified by a method of VOGEL [17] to obtain an azo-mitochondrial preparation. The protein concentration was adjusted to 2.4 g per 100 ml.

Experimental procedure

Closely bred Wistar rats were used.

Test group. Twenty-five rats were injected intraperitoneally at weekly intervals for six weeks with the azo-human kidney mitochondrial preparation. Each injection contained 3 mg protein in Freund's complete adjuvant.

Control group. Ten rats were injected intraperitoneally at weekly intervals with bovine serum albumin (BSA). The frequency of injections and the amount of protein administered in Freund's complete adjuvant were the same as in the test group.

Urinary protein analysis. Twenty-four-hour specimens of urine were collected from each rat, in metabolic cages at regular intervals until the termination of the experiment at 20 weeks. The daily protein loss was determined by a modified Weichselbaum biuret technique [18].

Light microscopy. Blocks of kidneys were fixed in 4% formaldehyde in saline. 3—4 μ thick sections were cut and stained with haematoxylin and eosin, periodic acid-Schiff and the methenamine silver method.

Fluorescent microscopy. One kidney from each rat was wrapped in parafilm and stored at —20 °C. 4 μ thick sections were cut on a cryostat, fixed in 4% phosphate buffered formalin pH 7.2, and then stained using mono-specific antisera to demonstrate rat immunoglobulins, rat complement and BSA.

Electron microscopy. 1 cu.mm blocks of renal cortex were fixed in 1% osmic acid, dehydrated in ethanol and embedded in Epon. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined on an AEI EM 6B electron microscope.

Results

Urinary protein analysis

In the experimental and control groups, a transient proteinuria was observed around four weeks. In the experimental group (in all but 2 rats) a second phase of proteinuria developed from 8—10 weeks onwards and

became progressively elevated. The control group did not develop the progressive second phase proteinuria.

A graph of the mean protein excretion for both groups of rats is shown in Fig. 1.

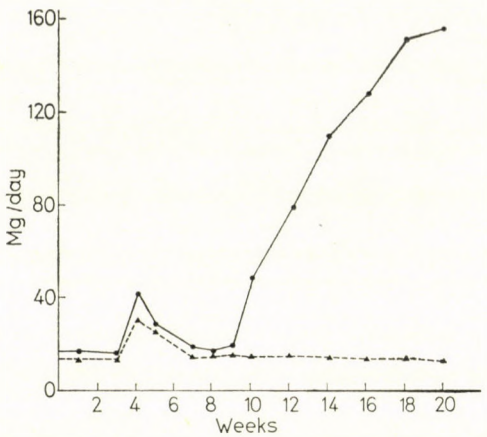

Fig. 1. Mean daily protein excretion in experimental (———) and control (- - - - -) rats

Light microscopy

All test group rats except the two animals without proteinuria showed moderate to severe thickening of the glomerular basement membrane at the end of the experiment. In methenamine silver stained kidney sections, numerous silver positive projections were noted on the outer surface of the glomerular basement membrane (Fig. 2). No cellular proliferation of glomerular tufts was observed.

The proximal convoluted tubules showed an increase in the number of hyaline droplets and many contained protein casts.

No histological abnormality was observed in any of the control animals nor in the two test animals without proteinuria.

Fluorescent microscopy

In the kidneys of those proteinuric rats which were killed at the end of the experiment, autologous gamma globulin and complement were demonstrated in a beaded fashion in the glomeruli (Fig. 3). The aproteinuric rats had no such glomerular localized components.

In the control rats there was no glomerular fluorescence when tested for the presence of BSA, autologous gamma globulin and complement.

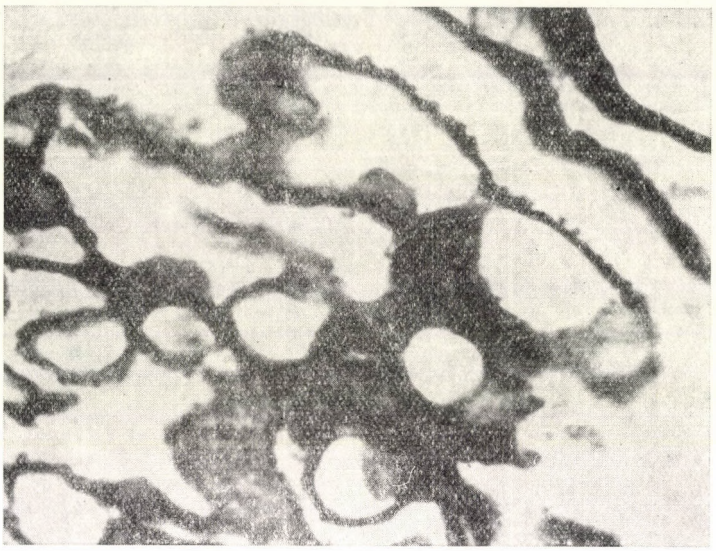

Fig. 2. Autologous immune complex glomerulonephritis. Portion of a glomerulus showing irregularly thickened glomerular basement membrane with numerous silver positive projections on its outer aspect. Methenamine silver, ×1100

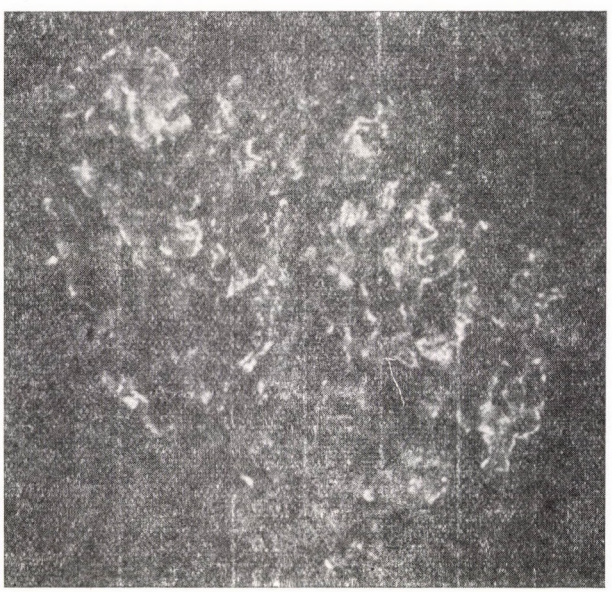

Fig. 3. Autologous immune complex glomerulonephritis. Autologous gammaglobulin is demonstrated along the glomerular capillaries in a beaded fashion. Fluorescent antibody technique, ×460

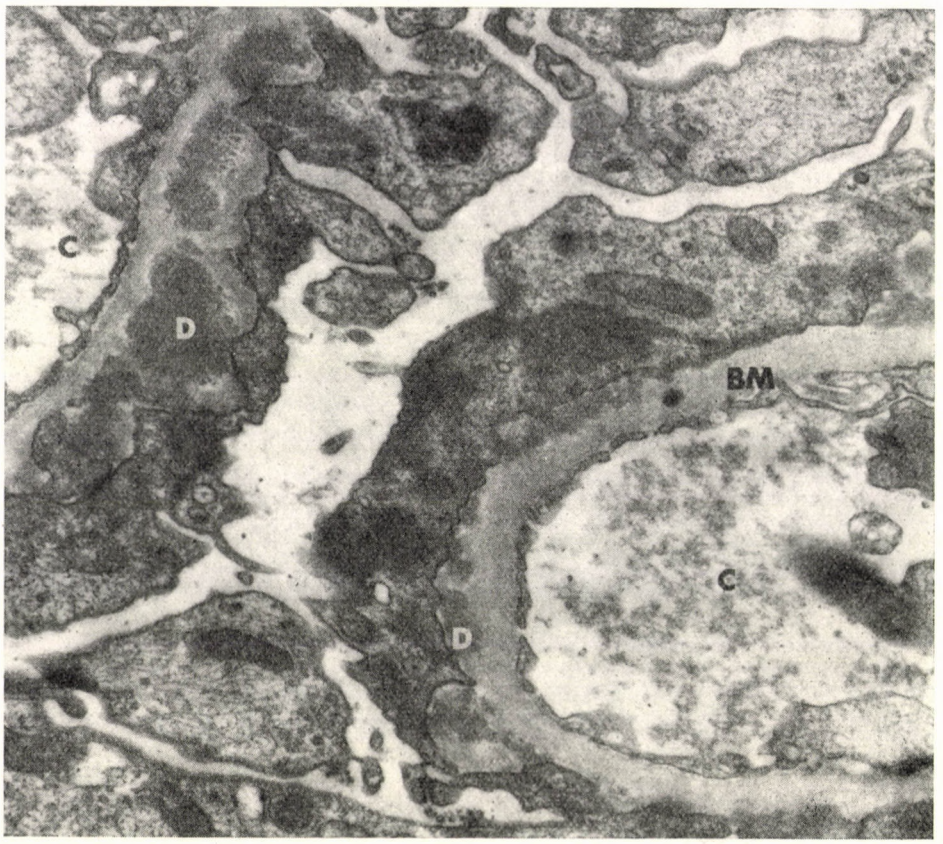

Fig. 4. Autologous immune complex glomerulonephritis. Portion of two glomerular capillary loops (C) showing numerous osmiophilic deposits (D) on the epithelial side of the basement membrane (BM). The foot-process layer is fused and the epithelial cell cytoplasm is osmiophilic adjacent to the deposits. EM, ×13,000

Electron microscopy

In the test group rats easily recognisable glomerular changes were observed. The glomerular basement membrane was irregularly thickened, with projections towards the epithelial aspect. Large subepithelial electron-dense deposits, partially or completely surrounded by basement membrane-like material were visible around most of the glomeruli. The epithelial foot processes were fused and the cytoplasm overlying the deposits appeared osmiophilic, often to the same degree as the deposits themselves (Fig. 4).

The tubules contained numerous protein absorption droplets (Fig. 5) and some of the mitochondria in the tubules were swollen. The tubular basement membrane showed no abnormality.

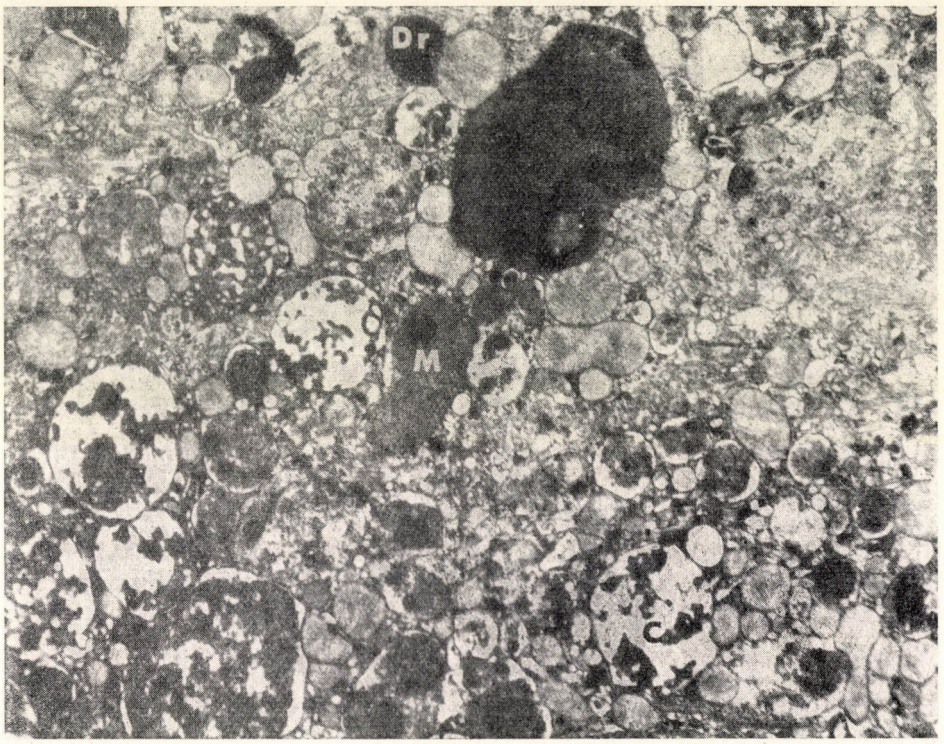

Fig. 5. Autologous immune complex glomerulonephritis. Portion of a proximal convoluted tubule showing protein absorption droplets (Dr), cytosomes (C), and altered mitochondria (M). EM, ×7000

In the kidneys of the two aproteinuric rats the only changes observed were occasional deposits on the epithelial side of the basement membrane. No ultrastructural change was observed in the kidneys of the control rats.

Discussion

AIC glomerulonephritis was induced in rats by repeated intraperitoneal injections of azo-human kidney mitochondrial preparation in complete Freund's adjuvant. The developing renal disease, as far as the proteinuria and morphology allowed to judge, was similar to nephritis produced by homologous renal antigen. The control rats manifested the temporary phase of proteinuria only and did not develop nephritis presumably because no circulating anti-kidney antibodies were induced by BSA.

It was shown by EDGINGTON, GLASSOCK and DIXON [14] that in heterologous renal antigen induced AIC glomerulonephritis, autologous renal tubular

antigen can be demonstrated in the glomeruli. Sometimes after the induction phase, autologous nephritogenic tubular antigen becomes available. The released antigen appears to be capable of initiating auto-antibody formation [19]. These auto-antibodies with the circulating nephritogenic antigen can form immune complexes in the presence of complement on the epithelial side of the glomerular basement membrane. It is likely that autologous renal tubular material becomes a continuous source of nephritogenic antigen. The

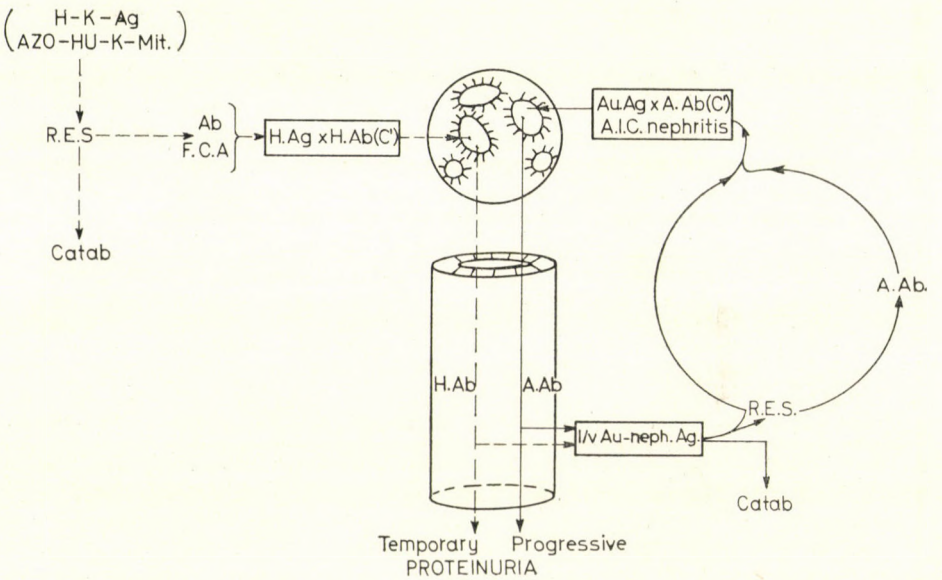

Fig. 6. Proposed pathogenic mechanism in autologous immune complex glomerulonephritis induced by heterologous renal antigen in the rat. The Figure shows the primary events (———) which lead to the release of autologous renal antigen. The cycle of events which maintain the release of autologous antigen during the chronic progressive phase of nephritis, the production of autoantibodies, and the formation of immune complexes in the glomeruli is shown on the right-hand side of the Figure (———)

chronic progressive phase seems to be maintained by a cycle of events which relies on autologous source of materials.

We have postulated that AIC glomerulonephritis is initiated and maintained by antibodies directed against the nephritogenic antigen in the proximal tubular cytoplasm [4, 15]. The following events, in agreement with our earlier assumptions, might contribute and eventually lead to the development of the chronic progressive kidney disease.

The azo-human kidney mitochondrial preparation in complete Freund's adjuvant causes the production of antibodies. These antibodies together with the circulating renal antigen produce a temporary phase of proteinuria early

in the experiment at 3—4 weeks, possibly due to a serum sickness type of lesion. During this period these cross-reactive antibodies (anti-human kidney mitochondrial antibodies) are able to pass through the glomeruli. They are either excreted or reabsorbed. Presumably some of the reabsorbed antibodies come in direct contact with the nephritogenic antigen in the proximal convoluted tubules. This immunological event may lead to the intravascular release of autologous tubular antigen. Auto-antibody may then be formed against this antigen. Autologous immune complexes comprised of auto-antibodies, auto-antigens, and complement might then be formed in the glomeruli. The cycle of events shown in Fig. 6 could maintain the release of nephritogenic antigen, auto-antibody formation and the immune complex deposition in the glomeruli.

The chronic progressive phase in membranous nephropathy in the human might be maintained by a mechanism similar to the presented cycle of events.

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EFFECT OF ADENOSINETRIPHOSPHATE (ATP) ON BLOOD FLOW AND CEREBRAL METABOLISM

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The response of cerebral blood flow and cerebral metabolism to the intravenous administration of 20 mg doses of ATP was studied in 30 subjects with cerebral ischaemia associated with cerebrovascular disease. Cerebral blood flow was measured by the counterflow-infusion, venous isotope dilution method with double puncture of the internal jugular vein. ATP was found to induce a substantial rise in cerebral blood flow with a fall in cerebrovascular resistance in a number of patients and a reduction in cerebral blood flow with a slight rise in cerebrovascular resistance in 6 patients. In general, neither cerebral blood flow nor cerebrovascular resistance was significantly affected by ATP. However, combined administration of ATP with noradrenaline in intravenous drip infusion resulted in a significant rise in cerebral blood flow together with a significant fall in cerebrovascular resistance. ATP, whether administered by itself or in combination with noradrenaline, failed to affect the cerebral uptake and utilisation of O_2 and glucose.

ATP plays a decisive part in cellular metabolism, being closely involved in energy turnover and glucose utilisation. Cerebral anoxia is known to induce a rapid fall in cerebral ATP concentration by interfering with ATP synthesis [1, 2]. On the other hand, it is also known that administration of ATP results in a vasodilation in various areas of blood supply. The data concerning the influence of ATP on cerebral blood flow and on cerebrovascular resistance is far from consistent. According to certain authors [3—6] in response to ATP cerebral flow increases while according to some others [7, 8] it remains unaffected. It has been shown earlier [9] that ATP administration is usually followed by a transient fall in carotid resistance whereas, owing to a simultaneous fall in blood pressure, carotid flow rises only transiently and to a slight degree.

In view of these facts it seemed rewarding to study the response of cerebral blood flow and of oxygen metabolism in ischaemic cerebral disease and to ascertain whether ATP could increase the reduced cerebral flow associated with such diseases.

Material and methods

In the study 30 subjects between 30 and 68 years of age (mean, 51 years) were involved. They all suffered from ischaemic disease of the brain (cerebrovascular encephalopathy, hypertensive encephalopathy), the presence of which was ascertained on the grounds of the history (transient spells of dizziness, visual disturbances, muscular paralysis, etc.) and the neurological investigation including EEG, carotid angiography, study of CSF, etc.

For the measurement of cerebral blood flow our counterflow, venous isotope-dilution method with double puncture of the internal jugular vein [10] was used. Cerebral blood flow was computed for one hemisphere, of the side of the punction. On the evidence of a large number of measurements, cerebral blood flow, referred to one hemisphere, corresponds normally to 425 $\pm$ 25 ml/min. Cerebral O_2 uptake was calculated from the quotient of cerebral blood flow and cerebral arteriovenous oxygen difference. O_2 in the blood of the femoral artery and jugular vein was measured with a Kipp-oxymeter. Cerebral glucose uptake was determined on the grounds of cerebral blood flow and cerebral arteriovenous glucose difference. Glucose was estimated according to Somogyi with a Hilgar electrophotometer. The respiratory quotient of the brain was determined on the basis of O_2-uptake and CO_2-release. O_2 and CO_2 content of the arterial and cerebral venous blood samples was estimated by Natelson's microgasometric procedure.

Arterial blood pressure was measured with Korotkoff's on the arm. Mean arterial pressure was calculated from the systolic and diastolic pressures, by Wiggers's formula, $P_m = P_d + 1/3\,(P_s - P_d)$.

Vascular resistance of the cerebral hemisphere was calculated from the cerebral perfusion pressure and from the blood flow of the cerebral hemisphere. In the measurement of cerebral perfusion pressure, cerebral venous pressure — internal jugular venous pressure — was also taken into consideration: cerebral perfusion pressure $= P_m - P_{jug}$.

The internal jugular venous pressure was measured in the bulb of the jugular vein with a Moritz—Tabora apparatus.

Cardiac output was determined by the dye-dilution principle, using 50 mg Evans blue.

In the first part of the study, determination of the basal values was followed by the intravenous administration of 20 mg ATP in not less than two minutes. Measurements of cerebral blood flow were performed at 10 min. (culmination of the effect), those of cerebral glucose, O_2-uptake and the cerebral respiratory quotient 10 min. and 15 min., respectively, after injection.

In the second part of the study the patients were given 20 mg ATP and 0.2 μg/kg/min. noradrenaline in drip infusion, in 10 min. Measurements were made prior to and after completion of the infusion. For statistical analysis, Student's two-sample t-test was used.

Results

Effect of ATP on cerebral blood flow

The effect of ATP on cerebral blood flow was studied in 11 cases of cerebral ischaemia. While mean basal cerebral flow referred to one hemisphere was very low, the vascular resistance was abnormally increased. Mean arterial pressure — the majority of patients being hypertensive — exceeded the average level. ATP induced a persistent reduction in blood pressure only in a few cases. A transient hypotensive effect was noted in every case, then blood pressure invariably returned to practically the initial value in a few minutes. The response to ATP of cerebral blood flow as well as of cerebrovascular resistance was inconsistent. While in five cases cerebral flow significantly increased and cerebrovascular resistance diminished, in the other six it was the cerebral flow which diminished while cerebrovascular resistance rose somewhat. Mean cerebral flow increased slightly and mean cerebrovascular resistance diminished slightly in response to ATP; these changes were not significant statistically. The last-mentioned changes are shown in Fig. 1.

The graph presented in Fig. 2 compares the parameters of two patient groups, one responding to ATP with an increase, the other with a reduction

in cerebral blood flow. As it can be seen, the two groups exhibited distinct differences in respect of the basal values for cerebral blood flow and vascular resistance. In Patients responding favourably to ATP (A) the cerebral blood flow was 270 ml/min., in those with an unfavourable response (B), 350 ml/min. It was remarkable to note the prevalence of symptoms indicative of peripheral arterial disease — intermittent claudication — in group B.

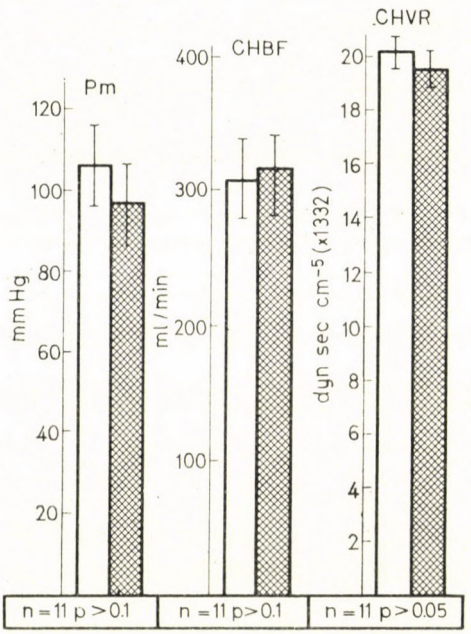

Fig. 1. P_m = mean arterial blood pressure. CHBF = cerebral blood flow calculated to one hemisphaerium. CHVR = cerebral vascular resistance calculated to one hemisphaerium. n = number of the examinations. p = probability level before and after ATP administration. I = Standard error of the means

Cerebral O_2 and glucose uptake, cerebral respiratory quotient and cerebral glucose/O_2-quotient remained largely unaffected by ATP, with the exception of a slight — non-significant — reduction in the respiratory quotient and an equally slight, non-significant rise in cerebral glucose/O_2-quotient demonstrable at 10 min. after ATP-injection, but no longer present at 15 min.

Effect of ATP and noradrenaline on cerebral blood flow

The response of cerebral blood flow to the combined administration of ATP and noradrenaline was studied in 11 patients. The essential results are presented in Fig. 3. Mean arterial pressure remained unaffected and cerebral blood flow increased from 350 ml/min. to 405 ml/min., a change attaining

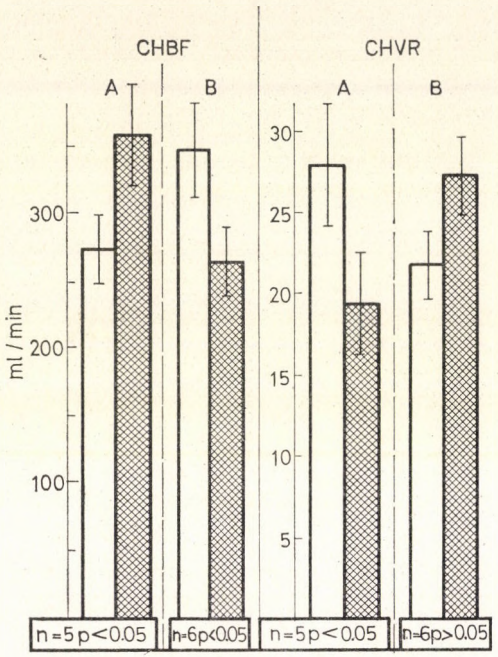
CHBF
CHVR
A
B
A
B
ml / min
300
200
100
30
25
20
15
10
5
n=5 p<0.05
n=6 p<0.05
n=5 p<0.05
n=6 p>0.05

Fig. 2

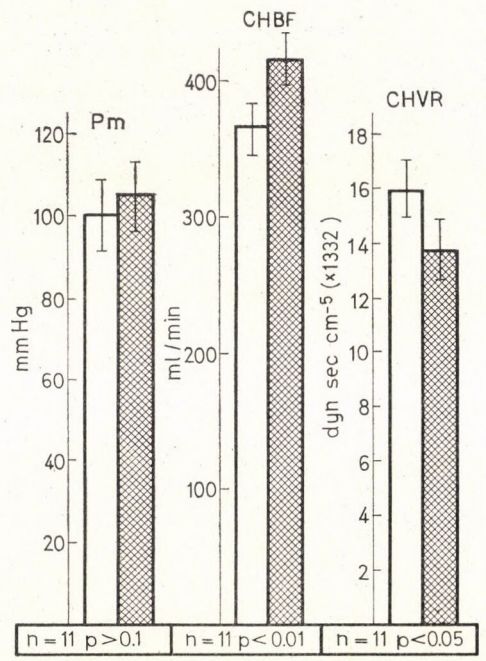
CHBF
Pm
CHVR
mm Hg
ml / min
dyn sec cm-5 (x1332)
120
100
80
60
40
20
400
300
200
100
18
16
14
12
10
8
6
4
2
n = 11 p > 0.1
n = 11 p < 0.01
n = 11 p < 0.05

Fig. 3

the level of significance in the same way as did the fall in cerebrovascular resistance. No negative response was noted.

ATP failed to affect the cerebral metabolism of O_2 and glucose, whether administered by itself or in combination with noradrenaline. No significant change in cardiac output was noted either in response to ATP itself or to its combination with noradrenaline. The same applies to the internal jugular venous pressure.

Discussion

It would be most desirable to be able to increase cerebral blood flow in conditions associated with cerebral hypoxia. However, cerebral circulation responds poorly to pharmacodynamic influences. This has two main causes: (1) cerebral blood circulation has a high degree of auto-regulation; (2) the cerebral vessels, compared to those of other regions, are sluggish to respond to stimuli. ATP and its derivatives have been recommended for reducing cerebrovascular resistance and to improve cerebral O_2-metabolism, the compound being a vasodilator producing a transient fall in blood pressure as well as vasodilation in the extremital and coronary vessels alike. Administration of ATP to laboratory animals was followed by a slight rise in the carotid and femoral flow despite a simultaneous, though slight, fall in blood pressure [9]. In our earlier human studies performed by the N_2O-method [9] we found that ATP by itself has but a slight stimulating effect on cerebral blood flow, but this effect was greatly enhanced if the accompanying transient fall in blood pressure was counteracted by the simultaneous administration of some potent vasoconstrictor.

In the present study noradrenaline was applied as the vasoconstrictor; its combination with ATP seemed advantageous, not only because of its capacity to counteract the transient hypotensive effect of ATP, but also because it has no direct influence on cerebrovascular tone. There is extensive experimental evidence [11—22] that noradrenaline affects only the tone of extracranial vessels while leaving cerebrovascular resistance practically unaffected. According to our own observations [9] noradrenaline by itself has no influence on cerebral blood flow.

The present study has yielded new evidence to the effect of ATP on cerebral blood flow. ATP by itself had no acute effect either on cerebral blood flow or on cerebrovascular resistance. However, if combined with noradrenaline, it induced a significant rise in cerebral blood flow with a parallel significant fall in cerebrovascular resistance.

A further point was the failure of cerebral O_2 and glucose uptake and utilisation to respond either to ATP or to ATP + noradrenaline. In syndromes associated with cerebral ischaemia the metabolism of the brain is greatly im-

paired as reflected by a poor cerebral O_2 uptake and by low values of the cerebral respiratory quotient as well as of the glucose/O_2 ratio. The last mentioned abnormalities are indicative of an impairment in the aerobic glycolysis of the brain. Administration of ATP left the metabolism of the brain unaffected, and even the enhancement of cerebral flow by ATP + noradrenaline failed to produce any favourable response. This indicates that, other things being equal, enhancement of cerebral blood flow by itself does not yet imply an improvement of cerebral metabolism.

The observation that in certain cases of cerebrovascular disease, ATP produces a prompt and marked fall in cerebral blood flow also deserves notice. The data presented in Fig. 3 would seem to suggest that cerebrovascular resistance fails to respond to ATP unless its original level has been excessively high. This is, however, at variance with the results obtained in the ATP + noradrenaline group where cerebrovascular resistance was not excessive, cerebral flow increased in response to the drug combination. It seems more probable that the frequent negative cerebral response to ATP is connected with its vasodilator property. ATP induces a transient fall in blood pressure and a general vasodilation, which often goes hand in hand with a reduction in cerebral flow. If coexisting abnormalities of other vascular areas, e.g. peripheral vascular disease, interfere with the production of general vasodilation, ATP may enhance cerebral blood flow.

The present results illustrate that the regulation of cerebral flow is inseparable from the general haemodynamic response and point to the possible benefits of the simultaneous administration of drugs affecting cerebral circulation from different sites of action.

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UNTERSUCHUNGEN DER NICHT VERESTERTEN FETTSÄUREN BEI EXPERIMENTELLER HYPERTONIE AN DER RATTE

Von

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Es wurde eine gaschromatographische Analyse des Musters der freien, nicht veresterten Fettsäuren (FFS) im Blutplasma bei zwei Modellen der experimentellen Hypertonie (nephrogen und neurogen-interorezeptiv) an der Ratte durchgeführt, und die Ergebnisse wurden mit denen gleicher normotoner Tiere verglichen. Bei hypertoner Belastung veränderte sich das FFS-Muster in charakteristischer Weise:

1. die gesättigten FFS, besonders die Palmitin- und Stearinsäure, erhöhten sich;
2. die ungesättigten FFS verminderten sich diametral zu den gesättigten, namentlich die Öl-, Palmitolein-, Linolsäure.

Die Veränderungen waren deutlicher bei dem nephrogenen Hypertoniemodell, annehmlich infolge der intensiveren Teilnahme des Renin-Angiotensin-Mechanismus.

Es ist bekannt, daß die nicht veresterten, freien Fettsäuren (FFS) im Plasma eine wesentliche Rolle als energetisches Substrat bei akuten und chronischen Belastungen im Organismus spielen. Die Kinetik der FFS-Freisetzung aus den Fettdepots in das Blutplasma, ihr Transport und die Oxydation verlaufen verschieden bei normalen physiologischen Bedingungen sowie bei Belastungen verschiedener Art, z. B. physischen und psychischen Stressoren wie auch bei chronischen Belastungen des Organismus auch im Sinne eines arteriellen hypertonen Syndroms. Es dominiert die Auffassung, daßunter diesen Bedingungen der beschleunigte Umsatz der FFS meistens mit einer Aktivierung des sympathischen Nervensystems einschließlich einer erhöhten Produktion von Catecholaminen gekoppelt ist, welche eine stimulierende Rolle auf die Adenylcyclase und 3',5'-AMP ausüben.

Neuere Untersuchungen von ORÖ und Mitarb. [12], HODGE und Mitarb. [7], VASSALLE u. Mitarb. [14], GEBBER und SNYDLER [6] u. a. zeigen, daß die Freisetzung von Catecholaminen teilweise ein Teil eines arteriellen interorezeptiven Baroreflexes ist. Eine elektrische Stimulation verschiedener submedullärer Hirnstrukturen, vorwiegend kreislaufaktiver Areale mit pressorischen, kardiovaskulären Reaktionen führen zu einer Inhibition von vagalen Komponenten und einer Stimulation des sympathischen Nervensystems. Man spricht von einer »Catecholaminerhöhung unter vagaler Kontrolle«. Es ist außerdem bekannt, daß eine erhöhte Lipolyse bei Elektrostimulationen des Sympathikus oder Hypothalamus unter einer koordinierten Kontrolle des Kortex vonstatten geht.

Methodik

Die heutigen chromatographischen Methoden sind imstande, die einzelnen FFS-Fraktionen im Plasma exakt zu trennen. Wir bedienten uns dabei der routinemäßigen Dünnschichtchromatographie und Gasflüssigkeitsverteilungschromatographie (Giede-Gaschromatograph mit dem Flammenionisationsdetektor mit entsprechender Reproduzierbarkeit), durch welche eine Untersuchung der einzelnen Fraktionen auch in minimalen Mengen möglich ist. Die gaschromatographischen Bedingungen waren folgende: Säulenabmessung 3 m × 4 mm. stationäre Phase 20% Äthylenglykolsuccinatpolyester auf Chromosorb W, 60—80 mesh, Säulentemperatur 180°C—190°C, Schleppgas Stickstoff, Durchfluß 2,6 l/Std., Empfindlichkeit 10^{-9}/10. Die Extraktion der Lipide aus dem Blutplasma erfolgte nach dem Chloroform-Methanol-Verfahren, ihre Fraktionierung mit Hilfe der Dünnschichtchromatographie, ihre Veresterung mit BE_3-Methanol. Es wurde die gaschromatographische Analyse des Fettsäuremusters im Plasma bei verschiedenen Hypertoniemodellen an der Albinoratte (Stamm Wistar, männliche Tiere von derselben Generation, 10 Monate, ca. 220 g) vorgenommen, wobei wir folgende Modelle praktizierten: das nephrogene Zellophankapselmodell (eine Modifikation von MEIER u. ZBINDEN [10] nach der Methode von PAGE [13]) und ein neurogen-interorezeptives Carotissinusmodell (nach KRIEGER [9]), und zwar jeweils an 20 Tieren. Als Kontrolle diente eine gleiche Anzahl normotoner Ratten. Der Blutdruck der normotonen Tiere zeigte 110 ± 9,5 mmHg, der der nephrogen hypertonen Tiere war 165 ± 12,5 mmHg und der der neurogen hypertonen 180 ± 12,5 mmHg. Die Messung des systolischen Blutdruckes erfolgte unblutig nach der Methode von FRIEBEL und VREDEN [5]. Nach 12stündiger Nahrungskarenz wurde den Tieren mittels Herzpunktion bei konstanten Versuchsbedingungen Blut entnommen. Als Antikoagulant verwendeten wir Natriumfluorid. Zur statistischen Verifikation unserer Ergebnisse benutzten wir den *t*-Test nach Student und die Korrelationsprüfung.

Ergebnisse

Tabelle I zeigt die Reihe der langkettigen gesättigten und ungesättigten Fettsäuren aus dem Gesamtspektrum. Deutlich erkennbar und statistisch verifiziert ist der Unterschied der summarischen Veränderungen der gesättigten Fettsäuren, die bei experimenteller Hypertonie eine starke Erhöhung zeigen. Die ungesättigten Fettsäuren zeigen eine diametrale Verschiebung, die ebenfalls signifikant ist. Bei dem nephrogenen Hypertoniemodell sind diese Unterschiede ausgeprägter, mit höheren Werten. Betreffs der einzelnen Glieder des Musters der FFS im Plasma bei verschiedenen Hypertoniemodellen konnten wir charakteristische gleichsinnige Veränderungen im Vergleich zu den Kontrollgruppen finden. Die gesättigten FFS sind erhöht, besonders die relativ kurzkettigen FFS, wie Laurin- und Myristinsäure, welche bei den normotonen Tieren in geringen Mengen zu finden sind. Das Niveau der biologisch wichtigen Palmitinsäure steigt an. Ebenfalls eine ausgeprägte Erhöhung zeigt die Stearinsäure. Die Verminderung der ungesättigten FFS betrifft vor allem die Ölsäure wie auch die Palmitoleinsäure und die essentiellen Fettsäuren.

Besprechung

Unsere Untersuchungen zeigten, daß bei Energiebedarf vorwiegend die ungesättigten Fettsäuren, insbesondere die Ölsäure, und sekundär die hochgesättigten, verwertet werden. Bei schweren Belastungen spielen bei

Tabelle I

C-Zahl	Normotonie %	Nephrogene Hypertonie %	Neurogen-interorezeptive Hypertonie %
I. Gesättigte Fettsäuren			
C_{12} (Laurinsäure)	1,0 ±0,2	4,8 ±1,1 ($p < 0,01$)	3,8 ±0,9 ($p < 0,01$)
C_{13} (Tridekansäure)	—	2,4 ±0,6	1,0 ±0,2
C_{14} (Myristinsäure)	3,5 ±0,5	8,6 ±1,4 ($p < 0,01$)	7,4 ±1,2 ($p < 0,01$)
C_{15} (Pentadekansäure)	—	2,5 ±0,4	2,3 ±0,4
C_{16} (Palmitinsäure)	35,2 ±3,9	40,6 ±3,8 ($p < 0,01$)	41,1 ±4,0 ($p < 0,01$)
C_{17} (Margarinsäure)	—	1,7 ±0,3	1,9 ±0,4
C_{18} (Stearinsäure)	4,8 ±0,7	11,8 ±2,1 ($p < 0,01$)	8,8 ±1,7 ($p < 0,01$)
C_{20} (Arachinsäure)	1,2 ±0,2	2,1 ±0,5 ($p > 0,01$)	2,0 ±0,5 ($p > 0,01$)
$\varepsilon =$	45,7%	74,5%	68,3%
II. Ungesättigte Fettsäuren			
$C_{14:1}$ (Myristoleinsäure)	1,5 ±0,4	—	—
$C_{16:1}$ (Palmitoleinsäure)	9,6 ±1,6	4,6 ±1,0 ($p < 0,01$)	5,5 ±1,4 ($p < 0,01$)
$C_{18:1}$ (Ölsäure)	24,6 ±2,2	13,3 ±1,9 ($p < 0,01$)	15,1 ±2,0 ($p < 0,01$)
$C_{18:2}$ (Linolsäure)	9,2 ±1,3	4,1 ±1,0 ($p < 0,01$)	5,8 ±1,2 ($p < 0,01$)
$C_{18:3}$ (Linolensäure)	6,1 ±1,1	2,5 ±0,6 ($p < 0,01$)	3,8 ±0,6 ($p < 0,01$)
$C_{20:4}$ (Arachidonsäure)	3,3 ±0,6	1,2 ±0,4 ($p \leq 0,01$)	1,5 ±0,5 ($p \leq 0,01$)
$\varepsilon =$	54,3%	25,5%	31,7%

dem energetischen Metabolismus die leichter mobilisierbaren und leichter synthetisierbaren gesättigten Fettsäuren eine Rolle. Das entspricht nach BLOCH [1] und WAREMBOURG u. Mitarb. [15] der Annahme, daß für das Leben im allgemeinen die Ungesättigtheit nicht entscheidend ist. Die Analyse des Musters der FFS bei experimenteller Hypertonie unterscheidet sich von den Veränderungen der FFS bei Noradrenalininfusion nach BÖHLE und Mitarb. [2], DESCOMPS u. Mitarb. [3], bei welcher eine Zunahme der biologisch wichtigen Fettsäuren, Palmitin- und Ölsäure, im Vordergrund steht. Die Stearinsäure, die wir bei unseren Modellen erhöht fanden, entspricht den Untersuchungsergebnissen von JÁKY u. Mitarb. [8], FELT und ŠTAJNEROVÁ [4], welche die gleichen Ergebnisse bei hypoxämischen Zuständen gefunden haben.

Die Resultate der Spektralanalyse der FFS im Plasma bei experimentellen Hypertoniemodellen fügen sich in das komplexe Problem des hypertonen Zustandes ein, wobei Kortex und Subkortex, die Catecholamine und andere neurohumorale und genetische Faktoren beteiligt sind. Bei der biokybernetischen Interpretation der Hypertonie als vermaschter Regelkreis der Blutdruckho-

möostase (nach NITSCHKOFF [11]) mit seinen Adaptations-, Kompensations- und Entgleisungsmechanismen spielen die FFS als Energielieferant eine Rolle.

Die Kinetik der Mobilisation und Utilisation der FFS, die verschiedene Länge ihrer Ketten und die verschiedenen Grade der Sättigung, zeigen eine gesetzmäßige Reaktion sowohl bei der normotonen Kreislaufsituation als auch bei den verschiedenen Formen der Hypertoniemodelle. Die graduellen Unterschiede der experimentellen Hypertoniemodelle zeigen deutliche pathologische Veränderungen in dem Gleichgewicht des Metabolismus, insbesondere bei der nephrogenen Hypertonie, bei welcher der Renin-Angiotensin-Aldosteron-Mechanismus von besonderer Bedeutung ist und in den Fettstoffwechsel eingreift.

Das gaschromatographisch analysierte Spektrum der FFS im Plasma bei experimenteller Hypertonie zeigt charakteristische Veränderungen, deren Wechselbeziehungen und deren Dynamik eng mit dem Gesamtstoffwechsel verbunden sind und die in Abhängigkeit zu der Belastung und den kompensatorischen biologischen Reaktionen des Organismus stehen.

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IODINE KINETICS OF THE ORGANISM UNDER THE INFLUENCE OF PHENYLBUTAZONE

By

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The effect of phenylbutazone on thyroid function has been studied by estimation of the coefficients of iodine uptake and mobilisation reflecting the iodine kinetics.

1. Sustained levels of the drug were found to produce a dose-related, but invariably significant inhibition of thyroid function.

2. Phenylbutazone applied as preliminary treatment left thyroid iodine metabolism unaffected.

3. In view of the absence of any increase in thyroid weight, it is not an enhanced TSH-production which accounts for the inhibitory effect.

4. Administration of the drug after completion of radio-iodine uptake results in a mobilisation of a "pseudo"-hyperthyroid type which, however, on the evidence of plasma activity studies expresses only the iodine released by the thyroid and thus the ratio of active to inactive fraction in the serum.

The phenylbutazone derivatives owe their world-wide use in rheumatic diseases and in other fields of medicine including gynaecology, to their antipyretic, analgesic and anti-inflammatory properties. They are believed to act as anti-inflammatory agents in a cortisone-like fashion by inhibiting the breakdown of cortisone [7, 8]. In view of the close interrelations between the various endocrine organs this activity cannot be restricted to any particular gland but possibly involves any other endocrine organ including the thyroid [12], as indeed there is clinical evidence that phenylbutazone derivatives inhibit the uptake of iodine by this organ [4, 11]. According to certain authors, the anti-inflammatory effect of the drug fails to assert itself in case of previous thyroidectomy, an observation pointing to the close involvement of the thyroid in the production of the effect [2, 14, 15, 16]. The analgesic effect of phenylbutazone is attributed by some other authors to the thyrostatic properties of the drug [1, 5].

The aim of the present study has been to ascertain to what extent phenylbutazone interferes with iodine metabolism and how this inhibition takes effect.

Material and method

The response of iodine metabolism to various factors is estimated on the basis of the changes in iodine-binding (α) and the mobilization (σ) coefficients.

In euthyroid subjects the value of α is roughly 0.1 h^{-1}, and that of σ, 0.005 h^{-1}, depending on the iodine content of food and of drinking water [3]. Both coefficients increase in the

case of hyperthyroidism and decline in hypothyroidism. The two coefficients can be computed from the ascending (iodine uptake) and from the descending (iodine mobilization) limbs, respectively, of the iodine retention curve on the basis of the differential equation [9, 10]

$$\frac{dT}{dt} = \alpha S - \sigma T$$

where T represents thyroid activity, S that of blood, α and σ the coefficients of uptake and mobilization, respectively.

Since the organic iodine binding coefficient can be only derived from that section of the iodine-retention curve where ^{131}I-mobilization is still negligible ($\sigma = 0$), the above equation has been referred to α by utilizing the values for 2, 4 and 6 hrs uptake [10] as follows

$$\alpha = c\left[T_2 \frac{(T_4 - T_2)^2}{(T_4 - T_2) - T_6 - T_4}\right] \lg \frac{(T_4 - T_2)}{(T_6 - T_4)}$$

where c represents a constant of 1.151 h^{-1}.

On similar grounds the mobilization coefficient of the retention curve can also be derived from the data of the descending limb of the curve where α is a negligible quantity

$$\sigma = \frac{\ln \frac{T_b}{T_a}}{t_a - t_b}$$

where T_a and T_b represent thyroid activity estimated at two arbitrary times (t_a and t_b), of the mobilisation phase of the iodine retention curve.

In the studies, male albino rats of 200 g average body weight, kept on Remington's iodine poor diet were used. Four groups of 30 to 40 animals each were formed. They were treated with phenylbutazone intraperitoneally. In each group 10 untreated animals kept otherwise under identical conditions served as controls. The active substance was administered in amounts ranging from therapeutic to toxic doses.

For determination of the coefficient

1) iodine retention curves were prepared by the use of carrier-free ^{131}I on the basis of the values found in vivo 2, 6, 24, 48 and 72 hours after administration, using a scintillation detector counter [6];

2) the amount of ^{131}I released into the blood plasma was estimated and, within this value, the ratio of organic to inorganic ^{131}I of serum iodine was determined. For serum iodine estimation the TCA-precipitation method was used and activity was measured in a well-type scintillation counter.

Results

In Group I, phenylbutazone was administered in doses of 50, 100 and 150 mg, each dose to 10 animals, the first dose being given simultaneously with ^{131}I and three further doses at 24 hr intervals. This scheme provided for sustained blood levels. The results are shown in Fig. 1 where the average iodine uptake curves of the animals are presented. The coefficients were then derived from these curves, with due consideration to the scatter.

Fig. 1 shows that prolonged phenylbutazone administration produced a dose related reduction of iodine uptake and mobilization in the animals.

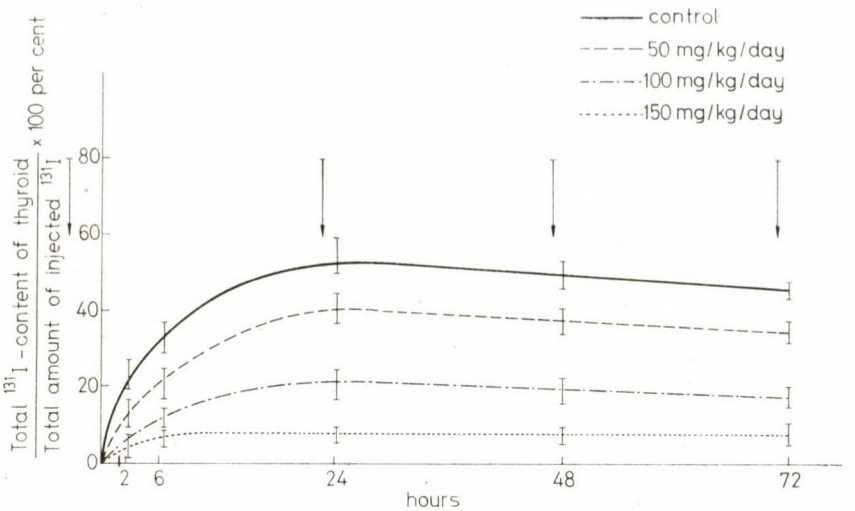

Fig. 1. ^{131}I retention curve of thyroid under sustained phenylbutazone level

This was also reflected by the differences of the α and σ values from the controls:

$$\alpha_{(c)} = 0.081\ h^{-1} \qquad \sigma_{(c)} = 0.0057\ h^{-1}$$
$$\alpha_{(50)} = 0.063\ h^{-1} \qquad \sigma_{(50)} = 0.0052\ h^{-1}$$
$$\alpha_{(100)} = 0.050\ h^{-1} \qquad \sigma_{(100)} = 0.0045\ h^{-1}$$
$$\alpha_{(150)} = 0.038\ h^{-1} \qquad \sigma_{(150)} = 0.0022\ h^{-1}$$

The reduction was significant statistically in every case ($p < 0.01$), with the exception of $\sigma_{(50)}$ where $0.05 > p > 0.01$.

The question whether the inhibitory effect of iodine uptake was due to a reduced formation of TSH or it was the consequence of an enhanced TSH-production representing a secondary effect of the drug as in the case of thiamazole, has been approached by two series of experiments.

1) Group II had a preliminary phenylbutazone treatment with doses of 25, 50, 100 and 150 mg daily for seven days and it was on the eighth day that they received the same intraperitoneal dose of ^{131}I as the others serving as controls. The iodine retention curve subsequent to treatment is shown in Fig. 2; it shows that the pretreatment did not influence the turnover of iodine, either in its uptake or in its mobilization phase, to an extent that might have affected ^{131}I uptake 24 hours later, i.e. at the time of ^{131}I administration. The toxic dose, which in 24 hours reduced the iodine retention capacity, induced convulsions in 50% of the animals.

2) In order to rule out a possible involvement of an inhibitory mechanism of the thiamazole type, the animals of Group III were given *A*: phenyl-

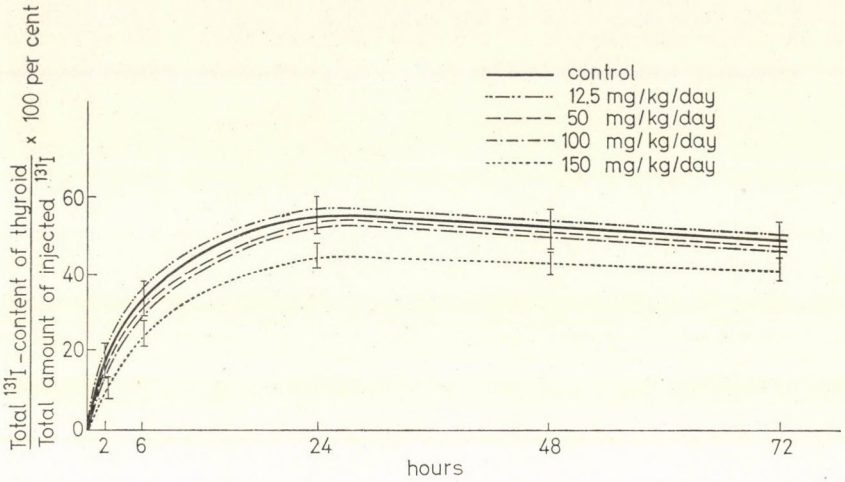

Fig. 2. ^{131}I-uptake after phenylbutazone pretreatment

butazone, *B*: thiamazole, *C*: the combination of these two drugs. After three weeks treatment, the thyroid was removed and the masses were compared. Results are summarized in Table I.

From the two series of experiments it clearly emerged that, unlike in the case of thiamazole, it was not an enhanced secretion of TSH which accounted for the inhibitory effect of phenylbutazone. This was confirmed first

Table I

Changes induced in thyroid weight by phenylbutazone, thiamazole and their combination

Animal groups	Average thyroid weight, mg	Scatter, mg	*t*	*p*
Untreated	17.90	±1.17	—	—
Phenylbutazone	13.66	±0.32	7.72	0.001
Thiamazole	50.92	±7.15	13.99	0.001
Phenylbutazone + thiamazole	39.94	±8.55	8.90	0.001

by the fact that at the end of the seven day pretreatment the intensification of iodine turnover consequent upon the increase in TSH secretion was not of a degree which could have led to a hyperfunction persisting even after the termination of treatment, and, second, by the absence of any increase in thyroid mass which would have resulted from an increased TSH secretion, an increase which, as illustrated by Table I, was in fact significant in the case of thiamazole treatment.

In the animals in Group IV treatment was begun 24 hours after the injection of ^{131}I and administration of the doses was repeated every 24 hours. In this group it has been studied how this treatment affected the steepness of the mobilization phase of the iodine retention curve, i.e. its σ. Fig. 3 compares the mobilization phase of the retention curves of the treated animals with that of the controls.

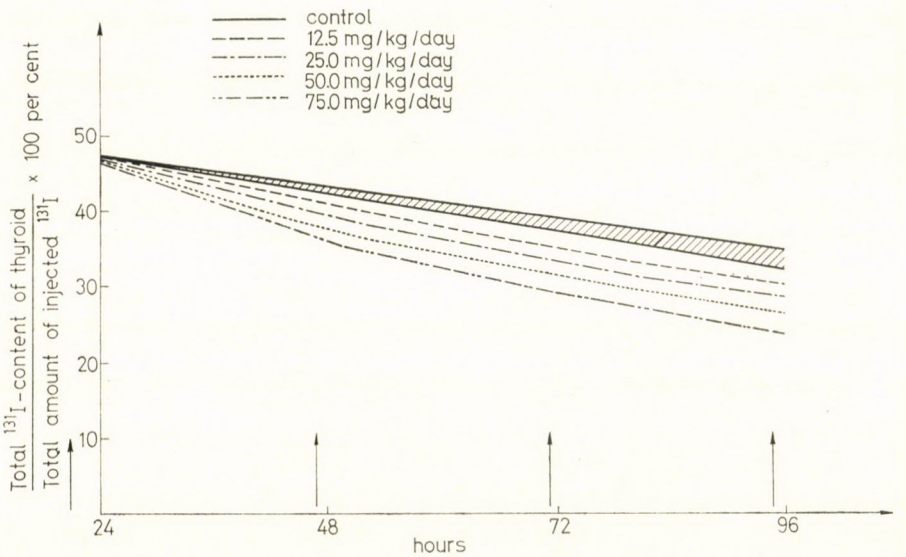

Fig. 3. **Phenylbutazone induced modifications of the mobilization phase of the iodine retention curve**

$$\sigma_{(c_1)} = 0.00475\ h^{-1} \qquad \sigma_{(25)} = 0.00695\ h^{-1}$$
$$\sigma_{(c_2)} = 0.00505\ h^{-1} \qquad \sigma_{(50)} = 0.00720\ h^{-1}$$
$$\sigma_{(12.5)} = 0.00625\ h^{-1} \qquad \sigma_{(75)} = 0.00800\ h^{-1}$$

Although the differences of the individual σ-values were not significant statistically, their increasing tendency was indicative of hyperfunctioning of the thyroid.

Since the results seemed to be at variance with the inhibitory effect noted in the other phenylbutazone series, the plasma ^{131}I level and the ratio organic to inorganic iodine was estimated in the sacrificed animals. Results are summarized in Fig. 4, which shows that as a result of treatment, the total plasma ^{131}I content diminished and the ratio organic to inorganic iodine underwent a dose related shift towards the inorganic phase. Thus, in contrast to the increase indicative of hyperfunction, the latter observation pointed to an inhibition of thyroid function.

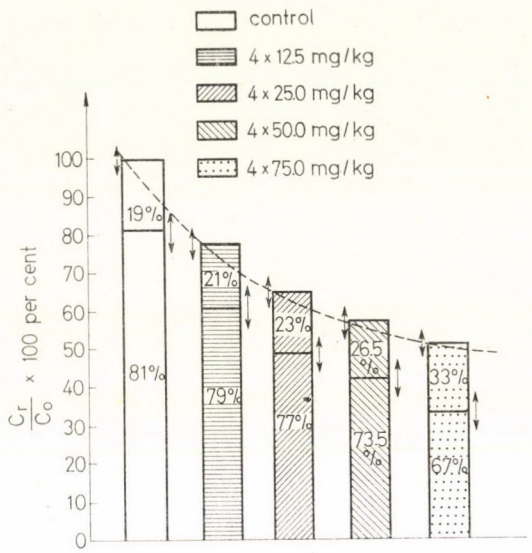

Fig. 4. Modifications of plasma ^{131}I and the ratio organic to inorganic iodine as a function of phenylbutazone treatment. C_0 = plasma ^{131}I in the controls; C_r = plasma ^{131}I in phenylbutazone treated animals

Discussion

From the inhibition of iodine uptake by the thyroid as well as from the reduction in total plasma iodine and in its organically bound iodine component, it may be inferred that phenylbutazone interferes with iodine turnover. This inhibitory effect asserts itself in the stage of iodine uptake, α having been significantly reduced. The low iodine uptake was followed by an impaired mobilization, as reflected by the significant reduction in σ. This type of inhibition may accompany a fall in the TSH-level. Since phenylbutazone exerts its cortisone-type effect by interfering with the breakdown of cortisone, it induces an increase in the cortisone level. The high cortisone level, on its part, then affects pituitary ACTH secretion. In view of the fact that the TSH is also produced by the pituitary, it may be assumed that, together with the production of ACTH, that of TSH is also inhibited, thus accounting for the changes in the uptake and mobilization coefficients characteristic of an impaired function, an interpretation supported by the results obtained in Groups II and III.

Inhibition of the mobilization phase ensued even if treatment was not started until iodine uptake had been complete. In this case, the inhibitory effect of the drug was obviously confined to the uptake of inactive iodine and the ratio active to inactive iodine shifted in favour of ^{131}I. This was, however, certainly independent of any drug induced alteration of iodine metabolism.

It may be assumed that, as a result of treatment, the ^{131}I liberated in the course of breakdown of the iodine hormones having attained the periphery, is excreted through the kidney instead of being resynthetized into iodine hormones. This interpretation would be consistent with our figures concerning plasma ^{131}I and the ratio organic to inorganic iodine. The finding that the plasma ^{131}I levels exhibit a dose dependent fall may likewise be connected with an inhibition of resynthesis.

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HAEMODYNAMIC STUDIES IN POLYCYTHAEMIA VERA

II. RESPONSE OF PERIPHERAL BLOOD FLOW TO SYMPATHO- AND PARASYMPATHOMIMETIC AGENTS

By

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The response of peripheral blood flow to sympatho- and parasympathomimetic agents has been studied in 40 subjects with polycythaemia vera. The majority of the patients, regardless whether in remission or in relapse, revealed a vasospastic susceptibility with an increased reactivity to adrenaline. The change responds poorly, if at all, to therapeutic factors, unless the primary disease is treated with success.

It has been shown earlier [1, 2, 4, 5] that the reaction to vasodilators and to exposure to cold was changed in patients with polycythaemia vera. These studies have revealed profound haemodynamic alterations involving the entire cutaneous vascular system in the periods of remission and recurrence alike. The present study has been concerned with the effects of sympathomimetic and parasympathomimetic agents on the blood flow of the limbs and on the microcirculation of the skin in polycythaemia vera.

Material and methods

Studies were made in 40 patients with polycythaemia vera (18 males, 22 females, average age 52.4 years, the youngest being 34, and the oldest 76 years old). In 15 of the 40 patients the investigations were carried out once, in 20 twice and in 5 three times, thus making up a totality of 70 investigations which form the basis of the present evaluation. Fifty of the 70 investigations were performed during periods of relapse and 20 during remissions. The red blood cell count of the patients averaged 6,380,000/ml in the periods of relapse and 4,350,000 in those of remission.

Control values were obtained from 28 healthy individuals (15 males, 13 females).

The patients as well as the control subjects were examined at 23 °C room temperature. The tonoscillogram was recorded on the upper limbs with a Flesh-apparatus, subsequently a tracing was made with a finger-plethysmograph with the aid of a direct-writing cardiograph adapted to this purpose. The vessels of the nail fold and the rate of capillary flow were examined with a Leitz capillaroscope. Skin temperature was measured on the flexor surface of the forearm with a thermistor skin thermometer with 0.1 °C accuracy. Evaluation of the results was based on the mean of the figures thus obtained. The reaction to direct exposure to cold was studied by the procedure of Burckhardt; the forearm of the side to be examined was immersed in a water bath of 15 °C for five minutes after which the time necessary for rewarming was measured [7], i.e. the interval between the termination of the cold bath and the return to +25 °C of the skin temperature. A susceptibility to arteriolar dilatation was indicated if the rewarming time was shorter than 9 minutes and a susceptibility to arterial contraction, if it

was as long as 21 to 30 minutes. To examine reactive hyperaemia, the upper arm was raised and a pressure slightly exceeding the systolic arterial pressure was applied to it by means of a manometer cuff, then the limb was lowered to the horizontal position and 5 minutes later the pressure was released. Under normal conditions the skin of the contracted limb became bright red within five minutes. In patients with blood flow abnormalities this time was prolonged by some minutes.

The tests were repeated after subcutaneous injections of 0.5 mg acetylcholine, 0.5 mg adrenaline, 0.5 mg noradrenaline, 0.05 g ephedrine and 0.06 g synephrine, respectively. After drug administration, the arteriogram, the rate of capillary blood flow and skin temperature on the forearm of the examined side were checked at 5 minute intervals over 30 minutes, then the oscillation tests were repeated and rewarming time after direct cooling as well as the time of reactive hyperaemia were again determined.

Results

The tonoscillograms of subjects with polycythaemia vera in remission revealed diminished amplitudes as compared with those of healthy controls. A further reduction in amplitude as compared with those found in remission, was demonstrable in the periods of relapse (Fig. 1). None of the drugs studied had any significant effect on the oscillatory amplitude.

Similarly to the amplitudes on the tonoscillogram, those of pulse plethysmogram also exhibited a moderate reduction during remissions and a major one in the periods of relapse. Pulse-plethysmography, being a far more sensitive procedure than tonoscillography, is better suited for the demonstration of blood flow disorders affecting the most distal parts of the limbs (Fig. 2).

In an earlier report [4] we have described the distinctive features of the capillary bed in polycythaemia vera. However, to gain an overall view of the condition of the vessels in the present series and to be able to compare the capillaroscopic findings of the polycythaemic patients with those of healthy subjects, we have to use higher magnifications (Figs 3a, 3b). The microphotographs are of $\times 100$ enlargement. In the periods of relapse of polycythaemia vera the arterial and venous portions of the capillaries are far more apart than under normal conditions and have nearly equal lumina. In the periods of remission these abnormalities subside for the greatest part. A continuous follow-up allowed to note an excessive slowing of the flow so that some of the capillaries became packed with stagnant red blood cell masses. This explains why acrocyanosis, persistent sensation of cold and, at lower temperatures, acroparaesthesis and pains in the distal part of the limbs are prevalent in polycythaemia vera. Injection of acetylcholine left the parameters of capillary flow unaffected. Administration of adrenaline resulted in a distinct, that of noradrenaline in a marked reduction in the flow rate, noradrenaline even brought the flow to a transitory standstill in the greatest part of the capillary bed. Ephedrine had scarcely any effect on capillary flow. Slowing of the circulation of the intracapillary blood column consequent upon the administration of synephrine was similar to that produced by noradrenaline.

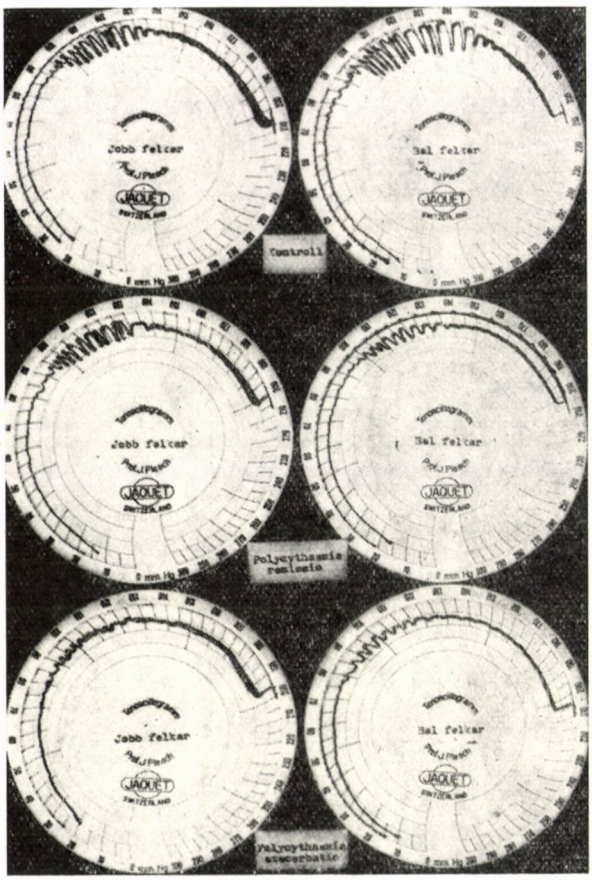

Fig. I

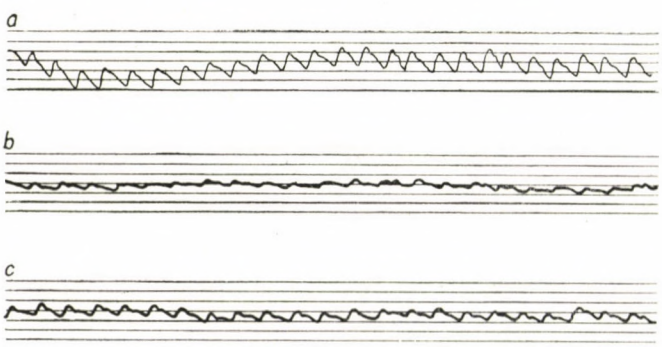

Fig. 2. a) Pulse plethysmogram of healthy subject. b) Pulse plethysmogram of patient with polycythaemia vera in relapse. c) Pulse plethysmogram of patient with polycythaemia vera in remission

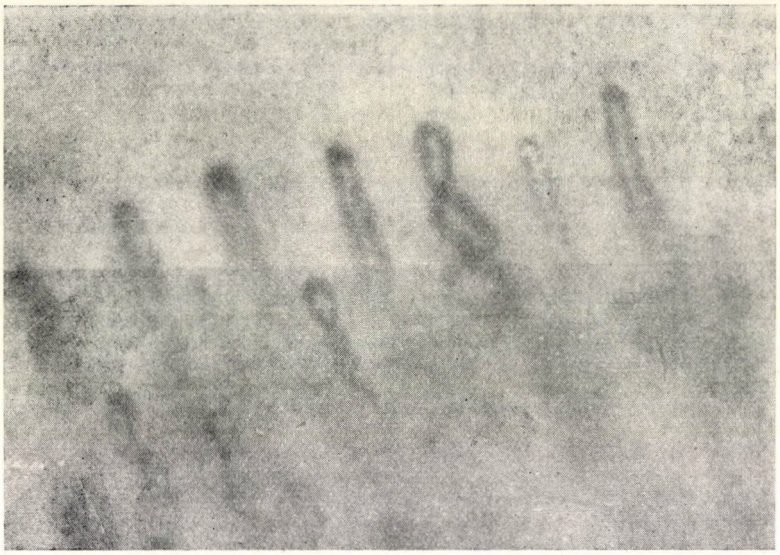

Fig. 3a. Capillaroscopic picture of polycythaemic patient in relapse

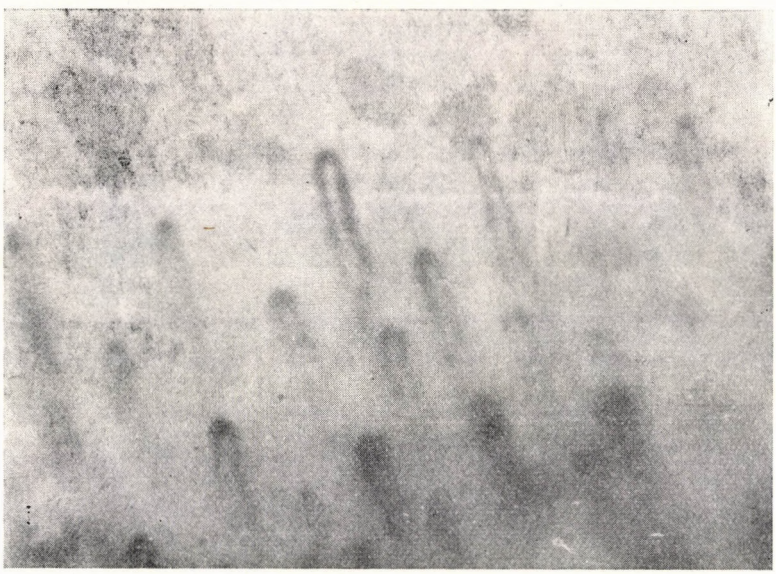

Fig. 3b. Capillaroscopic picture of polycythaemic patient in remission

Skin temperature, in comparison with that of normal subjects, was greatly reduced in every case of polycythaemia vera, in its periods of remission and recurrence alike. It remained unaffected by acetylcholine and was not significantly affected by ephedrine. However, it exhibited a significant fall in response to adrenaline, noradrenaline or synephrine.

Table I

Blood flow, skin temperature and rewarming time in healthy controls and in patients with polycythaemia vera in periods of relapse and remission after subcutaneous injection of vasoactive drugs

	Controls			Patients with polycythaemia vera					
				In relapse			In remission		
	Rate of blood flow cm/sec	Skin temp. °C	Rewarming time	Rate of blood flow cm/sec	Skin temp. °C	Rewarming time	Rate of blood flow cm/sec	Skin. temp. °C	Rewarming time
No drug	0.05 ±0.01	32.9 ±0.1	15′40″ ± 32″	0.005 ±0.001	28.4 ±0.1	37′10″ ± 1′	0.009 ±0.001	28.6 ±0.3	26′19″ ± 45″
0.5 mg acetylcholine	0.05 ±0.01	32.9 ±0.1	15′ ± 30″	0.005 ±0.002	27.4 ±0.1	37′5″ ± 55″	0.009 ±0.002	28.6 ±0.2	26′ 4″ ± 19″
0.5 mg adrenaline	0.03 ±0.01	31.2 ±0.3	16′55″ ± 37″	0.003 ±0.001	27.0 ±0.1	43′ ± 2′	0.007 ±0.002	27.2 ±0.1	29′ ± 1′
0.5 mg noradrenaline	0.03 ±0.01	30.7 ±0.4	17′12″ ± 25″	0.002 ±0.001	26.1 ±0.5	51′ ± 1′	0.005 ±0.001	26.9 ±0.4	36′ ± 1′
0.05 g ephedrine	0.04	32.1	15′55″	0.004	28.0	37′40″	0.009	28.0	25′55″
0.06 g synephrine	0.04	30.6	16′18″	0.002	27.6	58′49″	0.007	27.3	36′50″

Rewarming of the limb after direct exposure to cold was protracted in polycythaemia vera, and after administration of the drugs under study, particularly of noradrenaline, it was several times as long as normally. The changes in capillary flow rate, skin temperature and in the rewarming time consequent upon direct exposure to cold are shown in Table I.

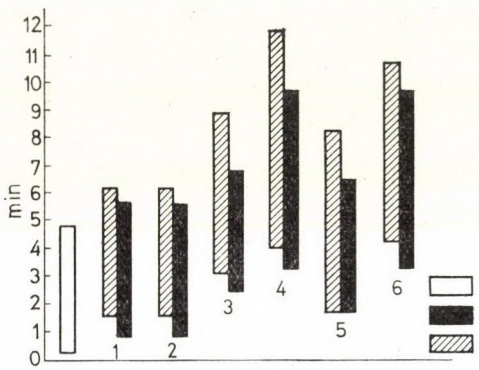

Fig. 4. Appearance and termination of reactive hyperaemia. ▭ Controls. Polycythaemia in relapse ▬; in remission ▨. 1 = No vasoactive drug; 2 = 0.5 mg acetylcholine; 3 = 0.5 mg adrenaline; 4 = 0.5 mg noradrenaline; 5 = 0.05 g ephedrine; 6 = 0.06 synephrine

Reactive hyperaemia appears slower and terminates later in patients with polycythaemia vera than in the controls. In the periods of recurrence of the disease the protraction is still more marked. While remaining unaffected by acetylcholine, it is greatly delayed both in production and course by adrenaline and ephedrine, and to an even greater extent by noradrenaline and synephrine, particularly in the periods of exacerbation of the disease (Fig. 4).

Discussion

The results of the present study indicate that polycythaemia vera is associated with a reduction in the rate of peripheral blood flow, as reflected by clinical signs such as acrocyanosis, low skin temperature, reduced amplitudes of tonoscillogram and pulse plethysmogram, slowing of capillary blood flow, protracted rewarming time, abnormal pattern of reactive hyperaemia as well as by subjective symptoms such as acroparaesthesis, pains in the distal part of the limbs etc. RATSCHOW [6] as well as HEILMAYER [3] refer to the occurrence of erythralgia in association with polycythaemia vera which is certainly a clear sign of abnormal vasomotor function.

The overreactivity of polycythaemic subjects to noradrenaline and synephrine also confirms the presence of vasomotor disturbances, in particular

of an angiospastic, less frequently of a vasodilatory susceptibility and accounts for the phenomena arising in response to variations of temperature, or to mechanical factors (overexertion or hanging of the hands), reactions which are demonstrable, though in an attenuated form, also during the periods of remission. The reduction in the rate of blood flow results from an increase in blood volume and viscosity. An adequate control of these abnormalities requires efficient therapeutic measures directed at the primary disease.

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LIPID FRACTIONS OF SERUM AND LIVER AFTER X-RAY IRRADIATION OF THE LIVER WITH 4000 R

By

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The lipid fractions in blood serum and in the liver have been studied after local administration of 4000 R to the liver. While in the first week after irradiation no significant change in the serum lipid components was demonstrable, the cholesterol concentration in the liver showed a significant decrease. This, however, proved reversible, the normal values being restored by the 42nd day. The phospholipids and esterified fatty acids remained unaffected in the liver, while in the serum the phospholipids increased significantly from the 7th, the esterified fatty acids from the 10th, and cholesterol from the 28th day onward. The fall in liver cholesterol during the first 42 hours after irradiation may be attributed to a direct injury to the organ. The rise in the serum lipid level at later stages suggests an involvement of additional factors.

Massive ionizing radiation induces changes in the serum lipid levels in mammals [8, 9, 11, 23]. The alterations of the individual lipid fractions have been found to vary from species to species. A fall in serum cholesterol has been noted in goats after fractional irradiation [14] and in rabbits after whole-body irradiation [24], while rats [15], guinea-pigs and dogs [6] exhibited an initial rise of the cholesterol levels.

From these divergent observations derived from whole-body irradiation of different animal species it would scarcely be possible to identify the tissue or organ the injury of which might be held responsible for the change in the serum lipid composition. In the present study, local irradiation of the liver was performed, i.e. of the organ which plays a decisive part in lipid metabolism. The aim was to ascertain whether such irradiation produced quantitative changes in the lipid fractions, and also to examine the changes in the tissue lipid level in the irradiated liver. Since the liver's radiosensitivity is a controversial issue and the organ has been considered radioresistant by certain authors [4, 13], we found it preferable to administer doses as large as 4000 R.

Material and methods

A total of 186 male Wistar rats weighing 280 $\pm$ 30 g was used. The radiation source was a THX 250 apparatus for depth therapy with a half value layer of 0.9 mm Cu, a focal-skin distance of 50 cm, and a dose rate of 97 R/min. The total dose was 4000 R measured on the skin surface. The field size of the area corresponding to the liver surface was 2.5 by

2.5 cm, with the exception of a part of its left lobe overlying the anterior surface of the stomach. In order to protect the stomach, this area of the liver together with the other parts of the body was shielded with 3 mm lead. Measuring the scattered radiation by means of $CaSO_4$: Mn thermoluminescent dosimeters, it was found to represent 1 to 3% of the direct radiation.

The animals were irradiated under pentobarbital anaesthesia (30 mg per kg body weight), and received normal rat chow and water ad libitum during the entire period from radiation until 48 hrs before processing, with the exception of the animals sacrificed on the first day, from which food was withdrawn 24 hrs before irradiation.

The individual groups were worked up 1, 2, 3, 5, 7, 10, 14, 21, 28, 42 and 63 days, respectively, after irradiation. Blood was withdrawn by heart puncture, likewise under pentobarbital anaesthesia.

A group of animals subjected to anaesthesia only was used as control, as it was found in preliminary tests that pentobarbital anaesthesia leaves the serum lipid fractions unaffected.

For estimation of the lipid fractions of the liver, specimens were taken from the middle part of the right lobe. The estimations were made at the same time of the day. Values were referred to 1 g wet weight, concentration of serum lipids was expressed in terms of mg per 100 ml. For evaluation of the results, Student's *t*-test was used.

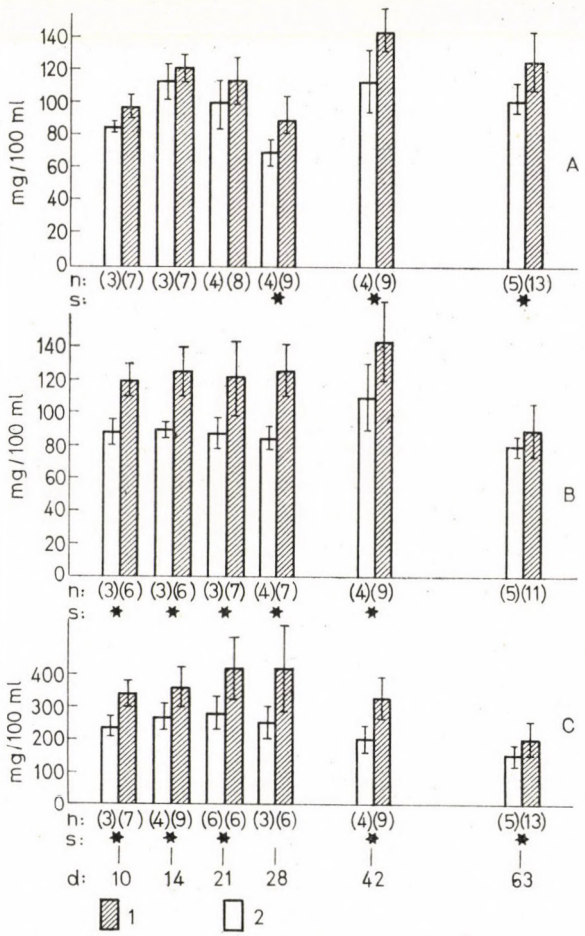

Fig. 1. A cholesterol; B phospholipids; C fatty esters in serum after local application of 4000 R to the liver. 1: Test group (anaesthesia and irradiation). 2: Control group (anaesthesia only). *: significant change (Student's *t* test)

The serum lipid fractions were estimated directly, those of the liver were extracted with chloroform-methanol (2 : 1) and the extract was salted out with 0.05 N KCl solution for the elimination of non-lipid components. Cholesterol was determined by the colorimetric method of ZAK [25] by sulphuric ferrichloride. For the determination of phospholipids the method of BAGINSZKI and ZAK [3] was used. The samples were treated with nitric acid or evaporated and the anorganic phosphate was estimated by colorimetry according to FISKE and SUBBAROW [10]. Fatty acid esters were determined in the form of hydroxamic acid as ferrihydroxamate, by means of CONNERTY's [5] hydroxylamine reagent used for ester linkages. The colour reaction thus produced lends itself for colorimetry.

Results

Serum. The cholesterol level remained unaffected until the 21st day after irradiation. From the 28th day to the end of the study there was a significant rise. The phospholipid concentration exhibited a significant increase on the 7th day and remained high until the 42nd day. By the 63rd day the

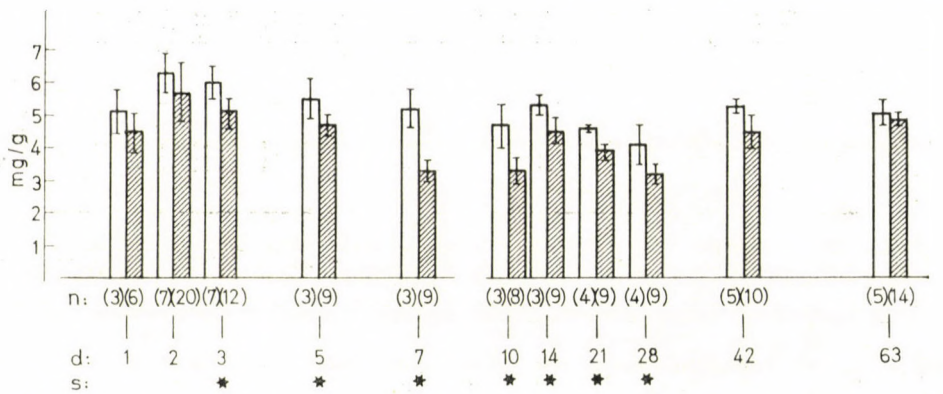

Fig. 2. Cholesterol content of liver after its local irradiation with 4000 R

value had normalized. The esterified fatty acids were found to increase at a relatively early time and the values remained high throughout. It was here that the fractions showed the highest elevation (Table I, Fig. 1).

Liver. The changes in the hepatic fractions were confined to cholesterol. From the 3rd to the 28th day, there was a fall in its concentration attaining its maximum between the 7th and 10th days. No change in the two other fractions was demonstrable at any time (Table II, Fig. 2).

Discussion

The pertaining data in the literature have mostly been obtained after total body irradiation.

Serum. A substantial increase in the cholesterol and phospholipid levels has been found after 1000 R whole body irradiation in rabbits [7]. A similarly

Table I

Serum lipid fractions after local application of 4000 R to the liver (mg/100 ml)

Day	Group	Cholesterol			Phospholipids			Esterified fatty acids		
		n	x	SD	n	x	SD	n	x	SD
	C	(4)	79	± 4	(4)	106	±16	(7)	196	±23
1	Rtg	(15)	84	±11 n.s.	(16)	102	±14 n.s.	(22)	222	±58 n.s.
	C	(7)	94	± 3	(7)	93	± 5	(5)	209	±23
2	Rtg	(12)	92	± 4 n.s.	(9)	101	±12 n.s.	(13)	214	±37 n.s.
	C	(7)	82	± 6	(7)	90	± 8	(4)	150	±11
3	Rtg	(10)	85	± 6 n.s.	(11)	92	± 7 n.s.	(11)	157	±24 n.s.
	C	(3)	102	± 6	(3)	103	±10	(3)	191	± 0
5	Rtg	(9)	110	± 8 n.s.	(8)	113	±23 n.s.	(9)	308	±71 n.s.
	C	(3)	97	± 9	(3)	97	± 4	(3)	202	±17
7	Rtg	(9)	107	± 8 n.s.	(6)	120	± 6 s.	(6)	252	±55 n.s.
	C	(3)	85	± 5	(3)	87	± 8	(3)	237	±30
10	Rtg	(7)	97	±10 n.s.	(6)	119	±10 s.	(7)	344	±40 s.
	C	(3)	113	±10	(3)	91	± 5	(4)	267	±35
14	Rtg	(7)	122	± 8 n.s.	(6)	125	±16 s.	(9)	360	±62 s.
	C	(4)	99	±15	(3)	88	±10	(6)	282	±48
21	Rtg	(8)	115	±12 n.s.	(7)	122	±24 s.	(6)	419	±92 s.
	C	(4)	70	± 7	(4)	85	± 8	(3)	249	±47
28	Rtg	(9)	99	±16 s.	(7)	126	±16 s.	(6)	417	±126 n.s.
	C	(4)	115	±22	(4)	111	±20	(4)	198	±40
42	Rtg	(9)	146	±14 s.	(9)	144	±24 s.	(9)	326	±60 s.
	C	(5)	103	± 9	(5)	81	± 7	(5)	152	±34
63	Rtg	(13)	127	±18 s.	(11)	90	±17 n.s.	(13)	202	±46 s.

Rtg: anaesthetized and irradiated group C: control group (anaesthesia only) n: number of animals x: mean
SD: standard deviation s.: significant change ($p < 0.05$) n.s.: non-significant

Table II

Liver lipid fractions after local application of 4000 R to the liver (mg/g wet weight)

Day	Group	Cholesterol			Phospholipids			Esterified fatty acids		
		n	x	SD	n	x	SD	n	x	SD
	C	(3)	5.1	±0.7	(3)	39	±1	(4)	46	±4
1	Rtg	(6)	4.5	±0.6 n.s.	(17)	35	±6 n.s.	(15)	43	±4 n.s.
	C	(7)	6.3	±0.6	(7)	38	±8	(4)	48	±4
2	Rtg	(20)	5.7	±0.9 n.s.	(19)	37	±5 n.s.	(15)	45	±3 n.s.
	C	(7)	6.0	±0.5	(7)	39	±4	(7)	47	±2
3	Rtg	(12)	5.1	±0.4 s.	(9)	38	±6 n.s.	(12)	46	±2 n.s.
	C	(3)	5.5	±0.6	(3)	39	±3	(3)	47	±2
5	Rtg	(9)	4.7	±0.3 s.	(8)	31	±7 n.s.	(9)	45	±2 n.s.
	C	(3)	5.2	±0.6	(5)	38	±7	(3)	45	±3
7	Rtg	(8)	3.3	±0.5 s.	(17)	37	±3 n.s.	(8)	42	±4 n.s.
	C	(3)	4.7	±0.6	(3)	33	±4	(3)	51	±2
10	Rtg	(8)	3.3	±0.4 s.	(7)	35	±4 n.s.	(8)	45	±7 n.s.
	C	(3)	5.3	±0.3	(3)	27	±4	(3)	55	±4
14	Rtg	(9)	4.5	±0.4 s.	(8)	29	±4 n.s.	(7)	48	±4 n.s.
	C	(4)	4.6	±0.1	(4)	30	±1	(4)	39	±3
21	Rtg	(9)	3.9	±0.2 s.	(8)	28	±5 n.s.	(9)	33	±3 n.s.
	C	(4)	4.1	±0.6	(4)	34	±3	(4)	37	±4
28	Rtg	(9)	3.2	±0.3 s.	(9)	29	±5 n.s.	(9)	36	±3 n.s.
	C	(5)	5.3	±0.2	(5)	40	±4	(5)	58	±2
42	Rtg	(10)	4.5	±0.5 n.s.	(10)	34	±5 n.s.	(10)	54	±5 n.s.
	C	(5)	5.1	±0.4	(4)	28	±2	(5)	45	±4
63	Rtg	(14)	4.9	±0.2 n.s.	(14)	27	±4 n.s.	(14)	42	±5 n.s.

significant rise was noted after 850 R whole body irradiation in rats [21]. Study of the fat depots has shown that fatty acids are successively released from them after whole body irradiation of rats [1].

Liver. An increase in the total lipid content has been reported in mice after 650 R whole body irradiation; the concentration of cholesterol and phospholipids remained unchanged [22]. On the other hand, a rise in the cholesterol contents has been noted in rats after 850 R whole body irradiation. 1000 R left the cholesterol level in rabbits unaffected while an increase was noted in phospholipids and neutral fats [7]. The central part played by the liver is indicated by the observation that the rise in the serum cholesterol level ensuing two days after whole body irradiation with 1000 to 2000 R in mice fails to appear after hepatectomy [17]. ^{14}C studies revealed an enhanced synthesis of cholesterol, phospholipids and neutral fats in the liver of animals after whole body irradiation [12, 19, 20].

After local irradiation, during the period from the 3rd to the 28th day it was only the liver cholesterol level which showed a decline, while the serum cholesterol was unaffected. The other lipid fractions failed to reveal any significant change until the 7th day after irradiation. No change in the phospholipid and fatty ester fractions was noted during the entire period of study.

The reduction in the cholesterol content of the liver must have been due to a direct radiation injury which, however, left the serum lipid fractions unaffected in the early stage. The changes in the serum lipids, thus the increase of the fatty esters on the 10th and of cholesterol on the 28th day are signs of a delayed reaction which, by lacking any parallelism with the changes in the liver lipids, does not seem to be the result of a direct radiation injury to the organ, though an enhanced lipid synthesis in the liver may well have been involved in the alterations.

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NEUROTOXIC EFFECT OF METHOXY-ETHYL-MERCURY-CHLORIDE

By

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The nervous effects of methoxy-ethyl-mercury-chloride have been studied in albino rabbits. Conduction velocity of the sciatic nerve was determined in 18 unanaesthetized rabbits placed in stocks. Following the oral administration of 6.0 and 0.6 mg/kg of MEMC daily over periods of 8 and 10 weeks, the conduction rate of the sciatic nerve was depressed to 57% and 40% of the control values, respectively. This was associated with a demyelination of the fibres of the sciatic nerve as revealed histologically.

Organic mercurial compounds, similarly to mercury and inorganic mercurial substances, are nephrotoxic and cause at the same time severe central nervous symptoms [1]. The histological observations of HAY et al. [8] and HUNTER et al. [9] have shown that the cerebellar Purkinje cells as well as the spinal motoneurons undergo vacuolar degeneration following ethyl-Hg-chloride and methyl-Hg-phosphate intoxication in man; these changes might be responsible for the characteristic clinical features of poisoning, the so-called Minimate Disease, described by TAKEUCHI [17], TSUBAKI [18] and KURLAND [11]. The mechanism by which organic mercury compounds (first of all methyl mercury) affect the central nervous system has not so far been elucidated [15, 16].

A characteristic compound of this group of substances is methoxy-ethyl-mercury chloride (MEMC), the fungicidal seed disinfectant. Observations from this laboratory [2] have shown that in the rat the compound, in addition to its nephrotoxic effect, causes characteristic central nervous signs such as tremor, ataxia, occasionally palsy of the hindlimbs. Also, MEMC has been shown to affect conditioned reflex activity in the rat [12].

To clarify the mechanism of the central nervous effects of MEMC, electromyographic studies were undertaken in rabbits.

Methods

A total of 86 experiments was undertaken in 18 full-grown male albino rabbits weighing 2.5 to 3.0 kg, by the method of KAESER and LAMBERT [10], using the stimulation technique described by EATON [3]. Conduction rate of the sciatic nerve was determined in the unanaesthetized rabbit, using a DISA 2-channel electromyograph. The nerve was stimulated through the

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intact skin, using bipolar electrodes and a DISA Ministim equipment, with supramaximal stimuli of 1 c/s, 0.2 msec; action potentials were recorded from the gastrocnemius muscle using needle electrodes. To eliminate the error due to synaptic delay, stimulation was performed at two points, at a distance of at least 1 cm, according to the method of EATON. The distance was measured with an accuracy of 1 mm, and conduction velocity was calculated from the difference, in the latency of the two action potentials obtained when stimulating the two points.

Prior to the experiments, a study was made to reveal whether an injury of the sciatic nerve arising as a consequence of stimulation through the skin or the resulting tissue reaction affected the conduction rate in the nerve. Therefore in 6 rabbits the conduction rate was measured six times at weekly intervals, and after killing the animals the sciatic nerve was subjected to gross and microscopic examination. Within this period, no tissue reaction occurred, and there was no change in the conduction rate. Then the effect of daily 6.0 mg/kg of MEMC given orally in a capsule on the conduction rate of the sciatic nerve was studied. This treatment was continued throughout a period of 8 weeks, during which the conduction rate was determined every second week on the basis of 160 or 250 measurements of latency. The data are subjected to statistical analysis, using Student's *t*-test. In the second series of experiments, one-tenth of the above dose, 0.6 mg/kg, was given to 6 rabbits daily over a period of 10 weeks.

The animals were observed throughout the entire period of experiments and the appearance of toxic symptoms was recorded; survivors were killed by a blow on the nape. Mercury levels in the tissues were determined by the dithizone method and expressed in g per 100 g wet weight. In addition to the usual histological techniques, luxol-fast-blue, Nissl and osmium tetroxide staining were used to reveal the changes brought about in the central and peripheral nervous systems.

Results

Conduction velocity. Maximum conduction rate in the control rabbits and in the experimental rabbits prior to the administration of MEMC ranged from 23 to 25 m/sec. Latency varied between 1.1 and 2.0 msec, the amplitude of the action potential attained 0.5 to 2.0 mV (Fig. 1).

Conduction rate of the sciatic nerve was significantly depressed in the fourth week of treatment in the group receiving 6.0 mg/kg of MEMC daily, the average being 10 m/sec ($p < 0.05$). The shape of the action potential deflection did not change. However, the stimulus threshold was significantly increased, in some cases from 3—5 mV to 10—12 mV. Four animals of the group died of the poisoning. Thus, the conduction rate was estimated in only two rabbits in the 8th week of treatment. Characteristic neurological symptoms were not seen in any of the animals; a significant loss of weight was recorded. The administration of daily 0.6 mg/kg of MEMC resulted in a gradual decrease of conduction rate, which was 12.5 m/sec in the 10th week of treatment ($p < 0.02$; Fig. 2). The animals remained free of neurological symptoms and showed a normal gain in weight.

Mercury in tissues. As compared to those of the controls, the organs treated contained substantial amounts of mercury, irrespective of whether the animals had died spontaneously or had been killed at the end of the experiment. Even the brain of these rabbits contained mercury, while in the controls not even traces of mercury could be revealed in this organ (Table I).

As expected, the highest amount of mercury was found in the kidneys, the average in the third group amounting to 5.8 mg/100 g.

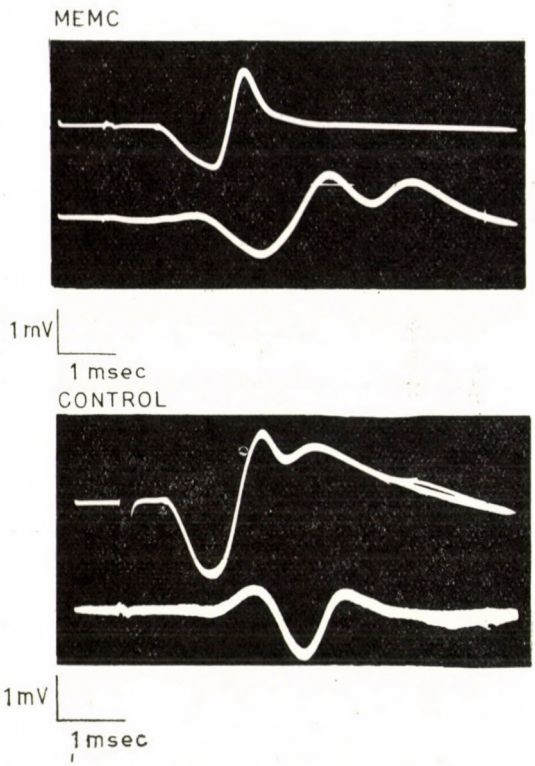

Fig. 1. Evoked muscle potential, photographed from oscilloscope. Rabbit No. II/1, 10 weeks MEMC treatment. Top tracing, experimental animal, 25 superimposed action potentials. Lower tracing, control animals, 25 superimposed deflections

Table I

Mercury, mg/100 g

No.	Doses mg/kg	Blood	Brain	Liver	Kidney
I/1–5	control	0.002	0	0.004	0.015
II/2*	6	—	—	2.80	10.0
II/4*	6	—	0.064	1.76	13.04
II/1	6	0.02	0.04	0.96	14.4
II/6	6	0.02	0.062	0.60	15.4
III/1	0.6	0.012	0.06	0.80	4.48
III/2	0.6	0.012	0.12	0.40	5.20
III/3	0.6	0.012	0.086	0.40	8.80
III/4	0.6	0.012	—	0.42	4.96
III/5	0.6	—	0.086	0.40	8.80
III/6	0.6	0.014	0.042	0.34	3.08

* died

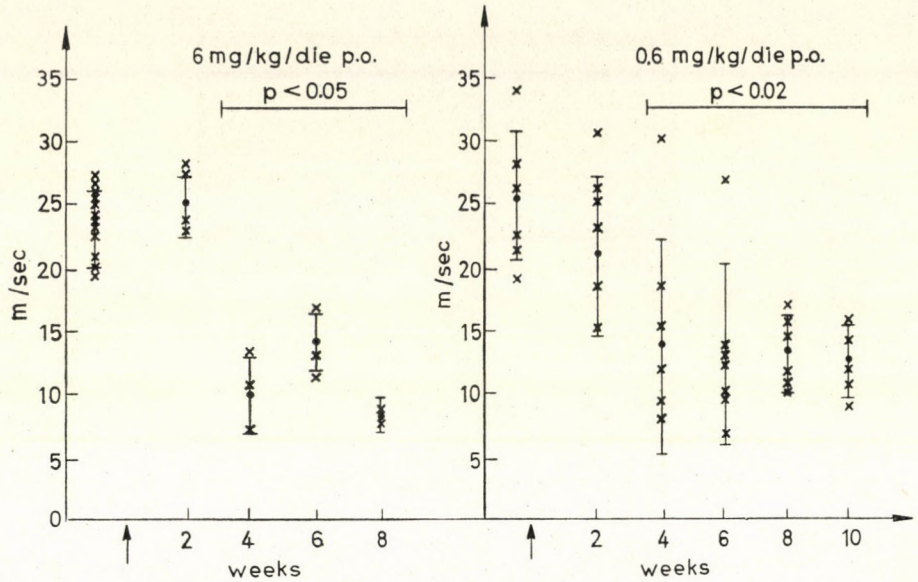

Fig. 2. Conduction velocity, as measured by the method of EATON, following administration of MEMC in two different doses. Abscissa, time from the onset of treatment (weeks). Ordinate, conduction rate (m/sec)

Histological studies. Both longitudinal and cross sections of the sciatic nerve showed marked demyelination with luxol-fast-blue and osmium tetroxide staining (Figs 3, 4). The other histological changes of the nervous system included degenerative alterations in the cerebellar Purkinje cells and spinal motoneurons (Figs 5, 6).

Discussion

Depression of the maximum conduction rate of the sciatic nerve in animals was recorded in polyneuritis caused by diphtheria and in lead and acrylamide poisoning [5, 6, 13, 14]. A decrease of the conduction rate was recorded also following acute thallium and phenol poisoning [7, 10] and exposure to mercury vapour [4]. In these experiments the change in the conduction rate of peripheral nerves was attributed to axonal degeneration and to demyelination. According to the observations of KAESER [10] fibres with a higher conduction rate showed signs of degeneration much earlier than fibres with a low conduction rate following acute thallium poisoning and in Wallerian degeneration due to transection of the nerve; nevertheless, it has been found that as far as a nerve can still be stimulated, it retains its conduction rate at almost normal values. In the present experiments, MEMC caused a significant depression of the conduction rate in the sciatic nerve of rabbits. When giving

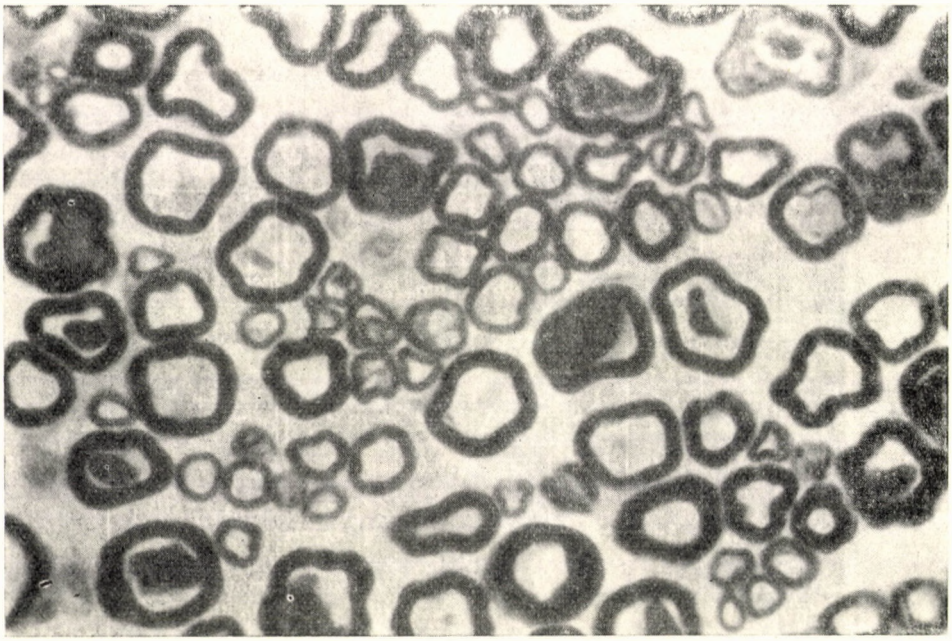

Fig. 3. Five-micron cross section of sciatic nerve, stained with osmium tetroxide. Contro

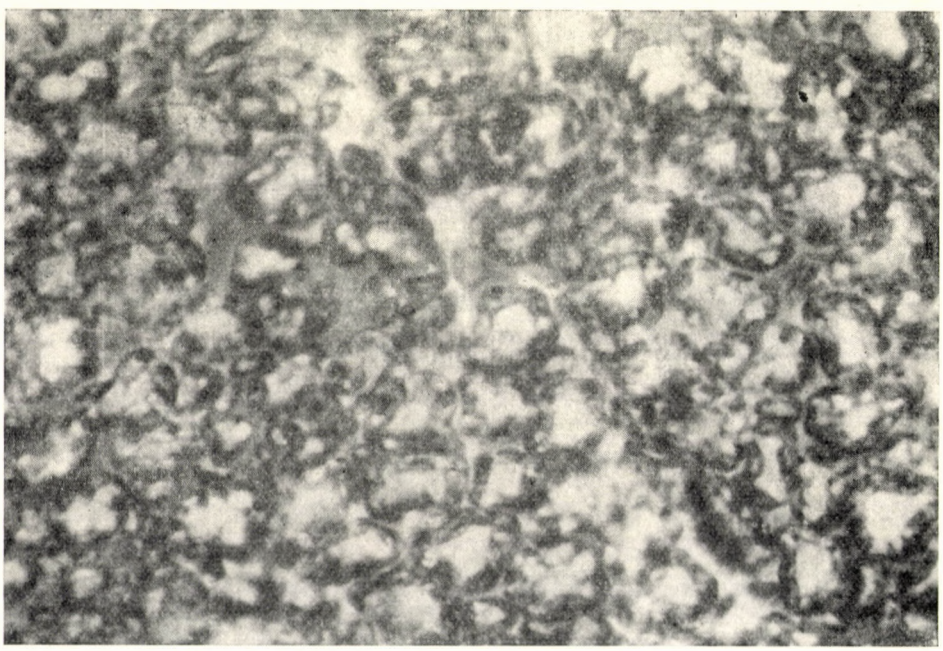

Fig. 4. Five-micron section of sciatic nerve, stained with osmium tetroxide. Rabbit No. II/6, 10 weeks MEMC treatment

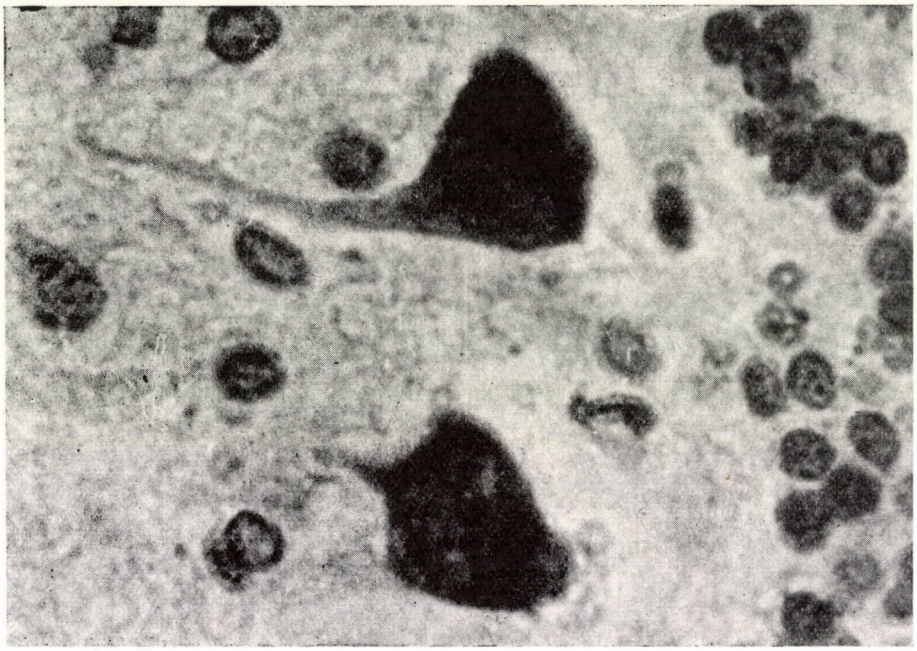

Fig. 5. Degenerative changes in cerebellar Purkinje cells. Nissl stain, ×400

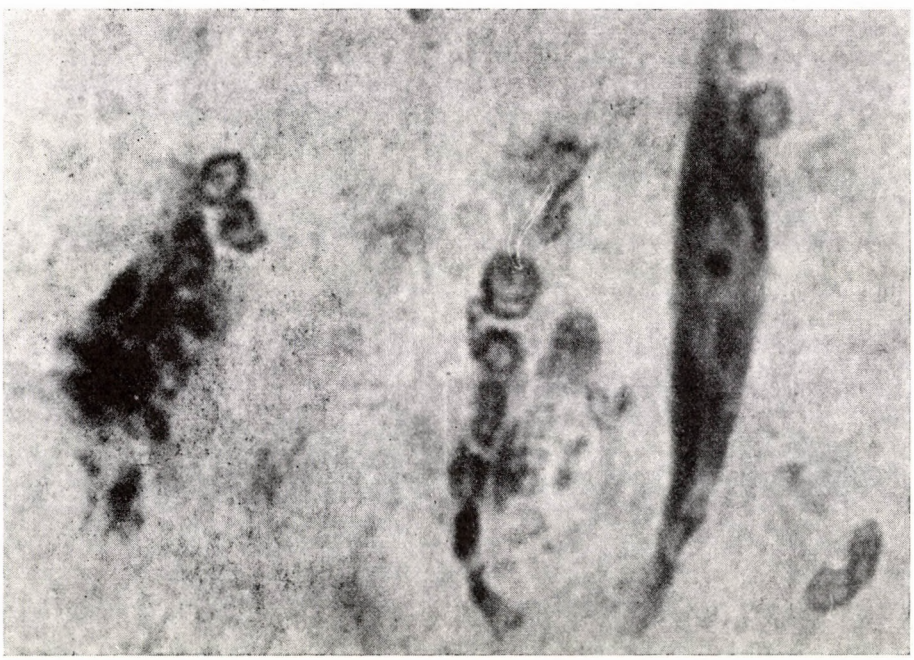

Fig. 6. Degenerative changes in anterior root of spinal cord. Nissl stain, ×450

doses of 6.0 mg/kg daily over a period of 8 weeks, the diminution of the conduction rate amounted to 57%, however, this dosage gave rise to toxic manifestations in some of the animals. Administration of 0.6 mg/kg daily, on the other hand, did not effect normal development and toxic symptoms failed to ensue during the 10-week treatment, despite the significant (40%) decrease of conduction in the sciatic nerve. Histological examination of the nerve has unequivocally shown that the change is due to a demyelination of the fibres in response to MEMC treatment.

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EXTRACELLULAR AND INTRACELLULAR FLUID COMPARTMENTS IN DOGS WITH INDUCED RENOVASCULAR HYPERTENSION

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Ligation of one renal artery and leaving the other kidney intact induces renovascular hypertension in the dog. The condition may be regarded as an analogue of the human clinical condition by sharing some of its essential features. In the chronic stage of this renovascular hypertension, thiosulphate space as well as plasma and blood volumes are significantly reduced. The reduction of the extracellular and intravascular fluid spaces is attributed to a maladjustment of the volume-sensitive receptors characteristic of the chronic stage of the condition.

As reported previously, ligation of one renal artery in the dog with the other kidney intact, arterial hypertension was induced (FEKETE 1967, 1970a), a condition which on the ground of its morphological features and various manifestations may be regarded as an analogue of the clinical syndrome of renovascular hypertension in humans. The experimental procedure allowed to study the syndrome in a species fairly close to the human being, moreover, to provide information concerning the unmanipulated kidney, since the ischaemic kidney ceases to function in 3 to 4 months as a result of progressive atrophy.

In chronic hypertension consecutive to ligation of one renal artery in the dog, a gradual restriction of the functional parameters of the unmanipulated kidney, arteriolar sclerosis and atrophy of the renal tissue ensue. In this condition, salt loading elicits abnormally high sodium losses and the venous blood of the operated as well as of the unoperated kidney exhibits an enhanced pressor activity, which the unmanipulated kidney maintains for a considerable period even after removal of the operated kidney (FEKETE et al. 1970a, b, 1971a, b).

In view of the close involvement of renin production in the regulation of fluid and electrolyte balance and of the fluid compartments under normal conditions (PEART 1965, GROSS et al. 1965, THURAU and SCHNERMAN 1965, VANDER 1967, 1970, WINDHAGER 1968, PESSINA 1970) an attempt was made to study in the dog the extracellular and intravascular compartments in renovascular hypertension accompanied by an increased renin activity and by a susceptibility to sodium loss. It was found that in the chronic phase of

experimental renovascular hypertension, at least 8 to 9 months after its production, both the extracellular and the intravascular compartments are significantly reduced.

Material and method

In the experiments, a test group of 24 mongrel dogs of either sex was used, and a group of 16 normotensive dogs (Group A) served as controls. In 24 dogs under pentobarbital anaesthesia from a midline incision the right renal artery was ligated, care being taken completely to suppress renal blood flow. The left kidney was left intact. The abdominal wound was closed under sterile conditions.

Measurement of blood pressure. Mean arterial pressure was measured directly by percutaneous puncture of the femoral artery with the aid of a mercury manometer in the alert state of the animals, these having been familiarized with the intervention. Arterial hypertension ensued in a few days after ligation of the renal artery. Blood pressure of these animals was checked at 20 to 30-day intervals throughout the entire period of observation.

Test groups. Since it has been noted in earlier studies that the individual parameters of renal function begin to decline when hypertension has been in existence for 6 to 8 months the fluid spaces were studied in 8 dogs (group B) in the 6th to 7th months, in 13 dogs (group C) in the 8th to 9th months and in 3 dogs (group D) in the 15th to 16th months of the state of hypertension. On every occasion the thiosulphate and intravascular spaces were determined, at least two estimations being made in each animal at intervals of 6 to 8 days.

The extracellular compartment was estimated on the basis of the thiosulphate space, by a single injection without collection of urine (BÁLINT 1958). In alert dogs having been familiarized with the procedure, under local anaesthesia, a plastic cannula was tied into the saphenous vein of one side. After withdrawal of a control blood sample, into a frontal leg vein 10 g sodium thiosulphate in 20 ml physiological saline was infused in 3 to 4 minutes so as to avoid the emetic effect of the substance (ROSS 1956). Blood samples were withdrawn 1, 5, 10, 15 and 20 minutes later and after centrifugation the plasma thiosulphate concentration was determined iodometrically. From the value thus obtained the spaces prevailing at the times of withdrawal (V) were determined on the basis of the equation $V = \frac{I \cdot v}{P}$, where I represents the thiosulphate concentration of the injected solution, P that of the blood plasma in mg/ml and v the volume of injected fluid in ml. In view of the rapid elimination of thiosulphate, the spaces measured at successive intervals showed an increasing tendency.

Plasma volume estimation. 20 min. after the administration of thiosulphate, 2 ml of a 0.5% solution of Evans blue was administered through the indwelling venous cannula. Blood samples were withdrawn four times at 10 min. intervals, centrifuged and the colour of the plasma was estimated by photometry. The logarithms of the plasma extinction values thus obtained were plotted on a coordinate system and from the value extrapolated for 0 min. the plasma volume, and with reference to the simultaneously determined haematocrit value the total blood volume was computed (BÁLINT 1958).

After completion of the measurements under local anaesthesia, the cannula was removed and the wound was closed under sterile conditions. The hypertensive animals as well as the controls were kept on the same normal standard diet supplemented with kitchen scraps. During the entire 7 to 16 months period of observation no changes in body weight were noted. For statistical evaluation of the results the two-sample t-test was used (FISCHER 1946).

Results

The figures and standard deviations ($\bar{x} \pm s_{\bar{x}}$) obtained in the 24 dogs with hypertension and in the 16 normotensive animals serving as controls have been summed up in Table I.

Blood pressure. Mean blood pressure was 126 ± 3 mm Hg in the controls, 183 ± 4 mm Hg in group B, 175 ± 6 mm Hg in group C, 176 ± 5 mm Hg

Table I

			Normal Group A	Hypertensive Group B	Hypertensive Group C	Hypertensive Group D
Number of animals			16	8	13	3
Number of experiments			32	17	26	6
Blood pressure, mm Hg			126 ± 3	183 ± 4	175 ± 6	176 ± 5
Body weight, kg			14.2	17.4	16.2	14.3
Thiosulphate space distribution	1 min.	liter	3.09 ± 0.10	3.55 ± 0.14	3.28 ± 0.13	3.23 ± 0.20
		per cent body weight	22.1 ± 0.4	20.7 ± 0.7 $p < 0.1$	20.2 ± 0.6 $p < 0.01$	22.3 ± 0.9 $p < 0.1$
	5 min.	liter	3.63 ± 0.10	4.16 ± 0.20	3.87 ± 0.16	3.44 ± 0.20
		per cent body weight	26.1 ± 0.6	24.4 ± 0.8 $p < 0.1$	23.9 ± 0.7 $p < 0.02$	24.0 ± 1.2 $p < 0.1$
	10 min.	liter	4.26 ± 0.10	4.97 ± 0.30	4.45 ± 0.20	3.83 ± 0.20
		per cent body weight	30.4 ± 0.6	28.6 ± 0.9 $p < 0.1$	27.3 ± 0.8 $p < 0.01$	26.7 ± 1.5 $p < 0.02$
	15 min.	liter	4.80 ± 0.10	5.44 ± 0.20	5.10 ± 0.24	4.34 ± 0.31
		per cent body weight	34.5 ± 0.7	32.5 ± 0.9 $p < 0.1$	31.3 ± 0.9 $p < 0.01$	30.2 ± 1.9 $p < 0.02$
	20 min.	liter	5.46 ± 0.20	6.77 ± 0.40	5.82 ± 0.25	4.94 ± 0.40
		per cent body weight	39.0 ± 0.8	38.6 ± 1.40 $p < 0.9$	35.3 ± 0.80 $p < 0.01$	34.4 ± 2.00 $p < 0.02$
Number of estimations (n) of plasma volume			35	18	25	6
Plasma volume		liter	0.85 ± 0.02	1.13 ± 0.10	0.84 ± 0.04	0.76 ± 0.07
		per cent body weight	6.0 ± 0.1	6.4 ± 0.5 $p < 0.1$	5.1 ± 0.2 $p < 0.001$	5.3 ± 0.3 $p < 0.05$
Haematocrit			41	38	44	39
Blood volume		liter	1.43 ± 0.04	1.82 ± 0.09	1.49 ± 0.07	1.26 ± 0.13
		per cent body weight	10.1 ± 0.2	10.3 ± 0.3 $p < 0.5$	9.1 ± 0.3 $p < 0.01$	8.7 ± 0.6 $p < 0.05$

in group D. The values for all test groups, as compared with those for the controls, were significantly increased ($p < 0.001$).

Thiosulphate space in group B with 6 to 7 months hypertension did not differ from the control. In group C with 8 to 9 months hypertension standing, the thiosulphate space was significantly less than in the controls ($p < 0.01$)

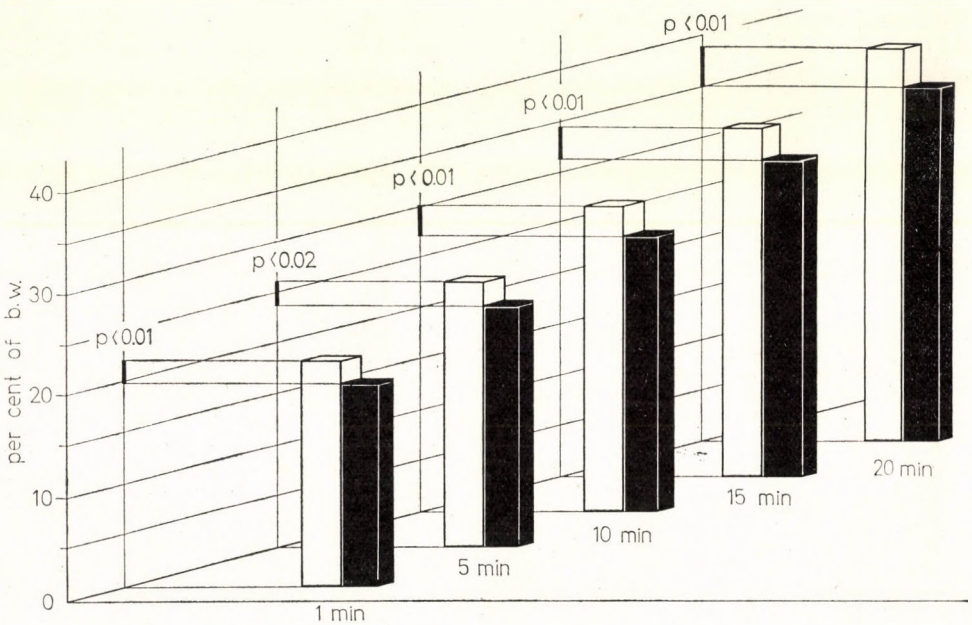

Fig. 1. Thiosulphate space in normotensive (empty column) and hypertensive (full column) dogs, in per cent of body weight, at 1, 5, 10, 15, and 20 min. after the thiosulphate administration. (The graph shows the data of groups A and C)

at each estimation. In group D comprising three animals with 15 to 16 months hypertension standing, the thiosulphate space was comparable with the control value at 1 and 5 min., but all subsequent measurements revealed significantly reduced values ($p < 0.02$), in absolute values as well as in the percentage of body weight.

Plasma and blood volume. Compared with the controls, plasma volume remained unaffected in group B and was significantly reduced in groups C and D. Total blood volume computed with reference to the simultaneously measured haematocrit value remained likewise unaffected in group B and was reduced in the more advanced groups.

Fig. 1 shows the result of 26 measurements of thiosulphate space at 1 to 20 min. in the 13 animals of group C (full columns), compared with the 32 measurements in the 16 animals of the control group A (empty columns).

The animals with chronic hypertension showed a significant reduction of thiosulphate space at all points of time.

Fig. 2 presents the values for blood pressure, plasma volume, haematocrit and blood volume of the same animal groups. In the hypertensive animals plasma and blood volume was significantly reduced as compared with the normotensive controls.

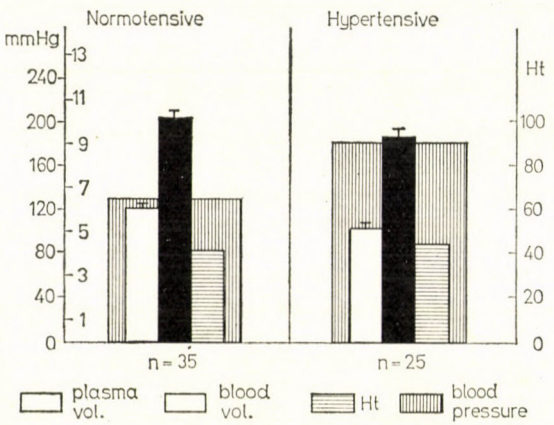

Fig. 2. Plasma and blood volumes and haematocrit value in normotensive and hypertensive dogs (groups A and C)

Discussion

In contrast to the plasma volume and the total fluid compartment which represent well-defined anatomical and physiological entities, the limits of the extracellular fluid compartment are not sharply defined. There are numerous substances, inulin, thiosulphate, mannitol, etc., which, on examination at suitable times, are found to be distributed over compartments of the body considered extracellular; the space thus estimated corresponds to 16 to 24% of body weight. However, more and more substances of this kind are found to be able to pass into the cells. Since the objective of this study has been to compare the particular fluid compartments, no reliable estimation of the extracellular space has been attempted.

Thiosulphate, a substance of prevalently extrarenal excretion, is particularly suited for experiments in dogs in view of its involving a single injection and no collection of urine. The thiosulphate space was found by CARDOZO and EDELMAN (1952) to correspond to 24.4%, by BECKER and JOSEPH (1954) to 24.1%, by FRANK and CARR (1955) to 22.5% and by RAISZ et al. (1953) to 24.7% of body weight. Similar values were obtained in the present study at 1 and 5 min. after thiosulphate administration.

For the estimation of plasma volume, Evans blue, a dye first recommended by GREGERSEN et al. (1950) was employed. The normal values range normally from 48 to 64 ml per kg body weight in dogs; GREGERSEN and STEWART (1939) and COURTICE (1943) obtained 56 ml/kg. REEVE (1947) pointed to species-related variations. In the present study, the procedure yielded plasma volumes of 60 ml/kg in normal dogs, a figure consistent with those quoted above. Since the haematocrit in the dog corresponds to that found in humans, the figures for total blood volume calculated on this basis are in general of the order of 100 ml/kg body weight.

As to the fluid compartments in hypertension, a 12% reduction in total blood volume has been noted by ROCHLIN et al. (1959). A reduction in plasma volume was observed by GORDON et al. (1967) in hypertensive human subjects. GREENE and SAPIRSTEIN (1952) ruled out the possibility of an expansion of plasma volume and of the extracellular space. TOBIAN (1960) found that the extracellular space was unaffected in patients in the 3rd to 5th months of hypertension. WINER (1958) reported normal findings in respect of all fluid compartments. CONWAY (1967) and LEDINGHAM (1953) noted in dogs with induced renal hypertension a transitory expansion of extracellular space which then normalized in 14 days. GROLLMAN and SHAPIRO (1953) demonstrated an increased extracellular space in Goldblatt-type hypertension, i.e. in dogs with a single kidney of restricted arterial flow.

In the present study the unilateral constriction of the renal artery, leading to a progressive atrophy of the organ, was followed by arterial hypertension. The changes were of a degree to involve a condition which then became stable in the course of three months following the intervention. After this period, the blood supply to the kidney, the intact tissue of which had shrunk to 10% of its normal substrate, was restricted to 10% of its original value (FEKETE 1967). Parallel with the development of hypertension, the unmanipulated kidney becomes involved in vascular lesions of degenerative character related in degree to the duration of hypertension and resulting finally in a narrowing of the arteriolar lumen and rigidity of the vascular wall, the renal tissue lacking any capacity of compensatory hypertrophy under these conditions. Gradual impairment of glomerular filtration rate, isosthenuria and compensatory polyuria ensue (FEKETE 1970a, FEKETE et al. 1971a). Though no permanent sodium loss of major degree was demonstrable in the dogs with induced renovascular hypertension, the presence of continued though slight sodium losses must be none the less assumed in view of the functional impairment of antinatriuretic and antidiuretic nature on salt and water loading (FEKETE et al. 1970b, 1971b).

The changes described in the foregoing became manifest around the eighth month of the chronic stage of hypertension. In order to determine whether these changes are associated with changes in the fluid compartments,

we have grouped the animals in a manner allowing to estimate these compartments prior to the manifestation of the other functional abnormalities (group B) as well as parallel with their progress (groups C and D).

It has thus been shown that in chronic hypertension, as long as no other functional abnormalities are present (i.e. for some 6 to 7 months following ligation of the renal artery), no change is demonstrable in the extracellular and intravascular fluid compartments. In the subsequent stage (group C) the thiosulphate space as well as the blood volume undergo a significant decrease (Figs 1 and 2) and, after hypertension had prevailed for 15 to 16 months (group D), parallel with the stabilization of hypertension and restriction of renal function, the hypovolaemia becomes permanent, the plasma is iso-osmotic, and its electrolyte composition corresponds to the normal values.

According to present knowledge, regulation of the electrolyte and fluid compartments falls to the kidney which copes with this task by various regulatory mechanisms, and the homeostatic mechanisms are mediated in all probability through the renin-angiotensin-aldosterone system (GAUER and HENRY 1963, THURAU and SCHNERMAN 1965, VANDER 1967, WINDHAGER 1968).

It is generally known that renovascular hypertension leads to electrolyte and fluid disturbances which find their most manifest expression in an increased sodium and water excretion by the kidney (COTTIER et al. 1958, EISINGER 1966, LOWITZ et al. 1968, BIRCHALL et al. 1958, HANENSON et al. 1963, SELKURT et al. 1965). The water and salt losing tendency is attributed by ULLMANN et al. (1965) to the vulnerability of volume regulation in the hypertensive organism, by MARTINO and EARLEY (1967) to a direct effect of perfusion pressure, by NICKERSON and SUTTER (1964) to an angiotensin-induced decrease in plasma volume, by VANDER (1963) to a direct inhibitory effect of angiotensin on tubular reabsorption, by DAVIS et al. (1966) to the DOC "escape" phenomenon, by RECTOR et al. (1968) to the formation of a substance inhibitory to tubular electrolyte absorption, by GORDON et al. (1967) to a decrease in plasma volume connected with sympathetic hyperactivity characteristic of chronic hypertension, by FEKETE et al. (1971a) to an increased renin activity exhibited by the venous blood of the operated and the unoperated kidney alike, by GIRNDT and OCHWADT (1969) to the enhanced medullary blood flow producing a fall in the osmotic gradient.

Assessment of the actual or possible involvement of the factors reviewed above allows to form a conception of the mechanism responsible for the volume relationships in renovascular hypertension. The enhanced production of renin-angiotensin in the early stage of hypertension maintains an increased arterial pressure due to vasoconstriction. The receptors of volume regulation situated in the vascular walls send out signals corresponding to a state of plethora (TOBIAN 1960) as a result of which the body gradually adjusts itself to the salt-losing condition. Impairment of tubular absorption associated with chronic

hypertension manifests itself in spontaneous polyuria as well as in a fall in the U_{osm}/P_{osm} ratio and goes hand in hand with a reduction in glomerular filtration rate (FEKETE 1970a). Though excessive sodium excretion under basal conditions (i.e. in the absence of any extra load) is not characteristic of this stage (COTTIER 1958), the osmotic performance of the kidney is reduced to 50% (BRODSKY and GRAUBARTH 1953) and loading with hypertonic saline solutions elicits excessive losses of water and sodium. Arteriolar sclerosis resulting from the continuous hypersecretion of renin-angiotensin is an additional source of hypovolaemia. The contraction of the intravascular compartment provides a renewed impulse for the production of renin, moreover, the arterioles on their part further increase their tone as a sign of adaptation to the diminished intravascular space. In a given stage, the permanent hypertension results in a contraction of the extracellular and intravascular fluid compartments, primarily as a result of a maladjustment of the extrarenal volume-sensitive receptors. The disturbances of sodium equilibrium are still masked by the readjusted homeostasis (FRIEDMAN et al. 1955). The spontaneous death ensuing after some length of time in dogs with chronic hypertension is attributed to haemodynamic factors resulting in a collapse of this compensatory homeostatic mechanism.

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ANTIBODY NATURE OF THE LONG-ACTING THYROID STIMULATOR

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A procedure suitable for the partition of antigen-antibody complexes was found to leave the activity of LATS-IgG unaffected. This is inconsistent with the claim that LATS is an antigen-antibody complex in nature.

While antibodies formed against the microsomal fraction of thyroid epithelial cells are more prevalent in LATS-positive than in LATS-negative cases, their titre in the blood plasma is unrelated to the plasma LATS level. Using column-chromatography for the separation of the slow and the fast IgG-fraction, LATS and CF were found only in the former whereas antithyroglobulin antibody in both fractions. This indicates that the various antibodies belong to different subfractions. The fact that LATS and microsomal antibodies belong to the same subfraction accounts for the difficulty of their separation.

The long-acting thyroid-stimulator (LATS) contained in the plasma of patients with Graves' disease is a substance identical with or strongly attached to IgG [13]. Its antibody character has, however, not been ascertained and there are indications that it may represent an antigen-antibody complex [11]. This has prompted us to study LATS under experimental conditions bringing about a dissociation of antigen-antibody complexes, and, should LATS prove to be an antibody, to examine its relationship with the antibodies to other thyroid antigens. It was also intended to examine the quantitative distribution of LATS and of the other antithyroid antibodies in the chromatographically separable, individual IgG fractions of different electrophoretic mobility.

Material and method

The antigen-antibody nature of the LATS-IgG complex was studied under experimental conditions which are known to cause the dissociation of immune complexes [18]. The separated LATS-IgG was dissolved in physiological saline and dialyzed for 24 hours at +4 °C against a 0.1 M glycine-HCl buffer pH 3.0, a procedure which results in an antigen-antibody dissociation without the risk of affecting the antibody. After centrifuging, the supernatant was dialyzed against 0.15 M NaCl at +4 °C for 24 hours by this procedure.

Four series of experiments were carried out in order to examine how far the thyroid-stimulating effect of LATS, in its untreated condition and after dialysis against the glycine-HCl buffer would be affected by activated charcoal. Assuming LATS to be an antigen-antibody complex, the antigen released during the dialysis would have to be bound by charcoal, in which case LATS would lose its thyroid-stimulating capacity. Therefore, in all four series, dialysis was followed by the addition of 20 ml of a suspension of 100 mg activated charcoal to the LATS-containing medium and incubation for 15 minutes. Otherwise the procedure was as described above. A similar test with LATS-IgG which had not been dialyzed served as control.

For the separation of slow and fast IgG, chromatography on DEAE-Sephadex-A-50 prepared according to BAUMSTARK et al. [1] was used [6]. 3.75 M ammonium sulphate was

added successively to 50 ml plasma at room temperature under continuous stirring up to a final concentration of 1.6 M. The mixture was left to stand at +4 °C for 1 hr, then centrifuged at 3500 r.p.m. for 30 minutes at −4 °C for the separation of the precipitate which was then dissolved in 3 ml distilled water and dialyzed against 200 ml distilled water for 48 hours while changing the water several times. Subsequently, dialysis was continued against 0.02 M phosphate buffer pH 6.6 for 24 hours. The precipitate was removed and the pseudoglobulin containing supernatant was transferred on 36×2.5 cm DEAE-Sephadex-A-50 columns pretreated with 0.02 M phosphate buffer. For the separation of the nonabsorbable slow IgG, elution was started with 0.02 M phosphate buffer pH 6.6 and 5 ml fractions were collected (Fraction I), then continued with 0.05 M phosphate buffer pH 6.5 for the separation of the absorbable fast IgG (Fraction II). For the estimation of protein in Fractions I and II an Unicam Spectrophotometer (280 mμ) was used. Then, the IgG-containing eluate was again dialyzed against distilled water for 24 hours and lyophilized. Before the estimation of LATS and of the antibody, the lyophilized protein was dissolved in physiological saline pH 7.0.

The IgG content of Fractions I and II was checked by immunoelectrophoresis.

LATS was estimated by the modified procedure of McKENZIE [4, 12]. The experimental data were analyzed on the basis of geometrical means. The reaction was considered positive if in the animals having been injected with the IgG-containing extract the blood radioactivity estimated 8 hours later exceeded 150% of the activity in the blood of the control mice having been given a 5% solution of albumin, and if the lower fiducial limit was in excess of 150.

The complement fixation test (CF) suitable for the demonstration of antimicrosomal antibodies and Boyden's passive haemagglutination test (TRC) for the demonstration of antibodies formed against thyroglobulin were described earlier [19]. The assays for antithyroid antibodies were regarded as positive if CF yielded a positive reaction at titres of 1 : 6 and TRC at 1 : 2500 or higher.

Results

Table I shows that the activity of LATS-IgG remained unchanged after dialysis against glycine-HCl buffer pH 3.0 and was not affected by activated charcoal (Table II).

Table I

Antigen-antibody nature of LATS

Examination of the thyroid-stimulating effect of LATS-IgG after dialysis against glycine-HCl buffer pH 3.0 and without this procedure. In brackets, the 95% fiducial limits

Serial number	Name	LATS-IgG	LATS-IgG + glycine-HCl
1	P.E.	299 (277—323)	251 (233—271)
2	J.K.	439 (415—463)	410 (388—434)
3	J.U.	173 (165—181)	170 (161—178)
4	M.D.	613 (562—668)	571 (523—622)
5	A.T.	273 (266—280)	258 (251—264)
LATS Standard 0.010 U		314 (288—341)	283 (260—308)

Table II

Thyroid-stimulating effect of LATS-IgG dialyzed against glycine- HCl buffer pH 3.0 and non-dialyzed, in the presence and in the absence of activated charcoal

Serial number	Name	LATS-IgG	LATS-IgG + charcoal	LATS-IgG + buffer	LATS-IgG + buffer + charcoal
1	Cz.I.	225 (216—234)	225 (216—234)	229 (219—238)	223 (214—233)
2	B.E.	424 (410—438)	416 (402—430)	415 (401—429)	411 (397—425)
3	D.E.	302 (293—312)	309 (299—318)	300 (291—309)	297 (288—306)
4	G.R.	298 (284—313)	267 (254—280)	281 (267—295)	266 (253—279)

The CF reaction serving for the demonstration of microsomal antibodies was positive in a significantly larger number of LATS-positive than of LATS-negative cases ($P < 0.1\%$). No such difference was found between the LATS-positive and negative groups with respect of TRC (Table III). On the other hand, no relationship between the plasma LATS level and the CF titre was demonstrable, as shown in Fig. 1.

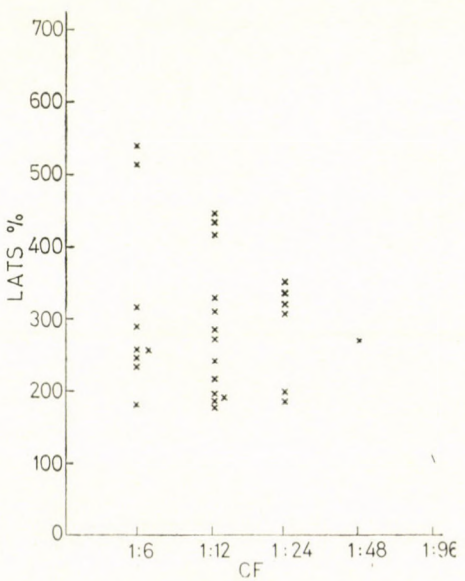

Fig. 1. Correlation between plasma LATS level and CF titre in patients with Graves' disease

Table III

Plasma LATS and thyroid autoantibody level in Graves' disease

	CF		TRC	
	positive	negative	positive	negative
LATS positive 69	30	39	13	56
LATS negative 70	10	60	13	57

Table IV

LATS and thyroid antibody contents of slow (Fraction I) and fast (Fraction II) IgG-fraction

Serial number	Name	Diagnosis	Fraction I			Fraction II		
			LATS	CF	TRC	LATS	CF	TRC
1	V.Gy.	Hashimoto	neg.	1 : 96	1 : 2500,000	neg.	neg.	1 : 2500,000
2	L.N.	,,	neg.	1 : 24	1 : 25,000	neg.	neg.	1 : 25,000
3	B.F.	,,	neg.	1 : 48	1 : 2500,000	neg.	neg.	1 : 2500,000
4	N.S.	Graves'	pos.	1 : 12	1 : 2,500	neg.	neg.	1 : 2,500
5	H.J.	,,	pos.	1 : 6	1 : 25,000	neg.	neg.	1 : 2,500
6	K.B.	,,	pos.	1 : 12	1 : 2,500	neg.	neg.	1 : 2,500
7	P.A.	,,	pos.	1 : 12	neg.	neg.	neg.	neg.
8	B.J.	,,	pos.	1 : 12	neg.	neg.	neg.	neg.
9	Sz.S.	,,	pos.	1 : 12	neg.	neg.	neg.	neg.
10	N.I.	,,	neg.	1 : 6	neg.	neg.	neg.	neg.
11	Gy.O.	,,	neg.	1 : 12	neg.	neg.	neg.	neg.
12	H.I.	,,	neg.	1 : 6	neg.	neg.	neg.	neg.
13	J.I.	,,	pos.	neg.	1 : 25,000	neg.	neg.	1 : 2,500
14	V.O.	,,	neg.	neg.	1 : 25,000	neg.	neg.	1 : 2,500
15	K.A.	,,	neg.	neg.	1 : 2,500	neg.	neg.	1 : 2,500
16	A.I.	,,	neg.	neg.	1 : 25,000	neg.	neg.	1 : 2,500
17	V.E.	,,	neg.	neg.	neg.	neg.	neg.	neg.
18	T.M.	,,	neg.	neg.	neg.	neg.	neg.	neg.
19	G.M.	,,	neg.	neg.	neg.	neg.	neg.	neg.

Table IV illustrates the presence of LATS and thyroid antibodies in the slow IgG I, and in the fast IgG II fraction. The first was eluted with 0.02 M (pH 6.6), the second with 0.05 M (pH 6.5) phosphate buffer. LATS and CF were detectable in Fraction I only. TRC was present in both fractions, though in Fraction II in slightly smaller amounts (Table IV).

Discussion

Since LATS cannot be separated from IgG, it is assumed to be an antibody directed against thyroid antigen [2]. It has also been suggested that it constitutes a complex formed by a product of thyrotrophic hormone character with IgG which accounts for its LATS-activity [11]. Antigen-antibody complexes are known to dissociate upon dialysis against buffers of low pH (pH 3.0). Since this procedure has been employed with success for the separation of insulin-antibody complexes [7, 10], we have studied its effect on the activity of LATS-IgG. The thyroid stimulating effect of LATS-IgG remained unaffected after dialysis against the glycine-HCl pH 3.0 buffer, a result which agrees well with the recent findings of Ochi and DeGroot obtained by a different procedure [16].

Our results may be interpreted as follows. *a)* LATS-IgG is not an antigen-antibody complex and therefore not dissociable; *b)* the procedure has failed to dissociate the antigen-antibody complex; *c)* the dissociation by the glycine-HCl buffer of the antigen-antibody complex may have been followed by its recombination before the estimation of LATS, an interpretation which does not tally with the finding that after dialysis the activated charcoal left the LATS unaffected, in other words it failed to bind the antigen which had been released from the complex.

Should LATS-IgG represent an antibody, its relationship to the other thyroid antibodies must be worth examining, the more so as on the evidence of earlier studies [5] positivity of LATS and CF often occur together, an observation confirmed by the present findings.

According to Torrigiani and Roitt [20] the antibodies formed against thyroid antigens belong to the 7S globulins and the overwhelming majority of the antithyroglobulin antibodies may be found in the IgG fraction [20]. These authors found antibodies of IgA type too in a small number of cases but, with the exception of one case, failed to demonstrate IgM antibodies. On the other hand Cruchaud and Juditz [3] noted various types of antibody (IgG, IgA, IgM) against thyroid cells in a diversity of combinations. LATS is inseparable from IgG, though its antibody nature has yet to be ascertained. It is an essential question how the antibodies formed against the individual thyroid antigens are distributed in the heterogeneous IgG.

Immunoelectrophoresis of IgG gives a precipitation band deeply extending into the zone of beta-globulins, as a sign that it combines various protein molecules different in mobility but identical in antigenic structure. Chromatography on DEAE Sephadex A-50 is well suited for the separation of the heterogeneous IgG-molecules [17]. Immunoelectrophoretic analysis of the chromatographic fractions shows that the IgG-fractions eluable from the DEAE-column with eluents of low molarity are of slighter mobility than those obtained with buffers of higher molarity. The electrophoretic heterogeneity of the IgG molecules shows a physiological heterogeneity too, since it seems that each of the individual IgG groups plays a particular part in the immune mechanisms. In the absence of cysteine, the fast component is fairly resistant to papain, in contrast to the slow component which is readily digested by papain [8]. The former group comprises the prevalent part of proteins belonging to the subfractions IgG_2 and IgG_4, and the latter group those of subfraction IgG_3 [9]. The proteins of subfraction IgG_1 have been found heterogeneous under such treatment.

It has been shown earlier [6] that the LATS-containing, non-absorbable IgG obtained by chromatography on Sephadex A-50 was of an electrophoretic mobility different from that of the other IgG, it being localized nearer to the cathode (slow fraction). This result was in conformity with that of MIYAI and WERNER [14] according to which IgG of substantial LATS content differs in respect of electric charge from the other IgG. On the evidence of our recent studies, while LATS and antimicrosomal antibody occur only in the slow fraction, antithyroglobulin antibody is demonstrable in the slow as well as in the fast fraction. This would seem to indicate that LATS and antimicrosomal antibodies prevalently belong to subfraction IgG_3, possibly to IgG_1, whereas the antithyroglobulin-antibodies are distributed over all four subfractions. Though LATS and antimicrosomal antibodies are often found together in Graves' disease and both seem to belong to the same IgG subfraction, yet it is certainly not against the same antigens that they have been formed. In Graves' disease, too, positive LATS and CF reactions are often dissociated, and in Hashimoto's disease antimicrosomal auto-antibody is usually demonstrable although LATS is absent from the plasma. The results of our studies none the less confirm the close relationship existing between antimicrosomal antibody and LATS, a relationship which follows from the fact that both belong to the same IgG subfraction. This might explain the difficulty of their separation. And, our findings indicate that the practical difficulty in identifying LATS by the radioimmune procedure based on a competitive binding [15] must be sought in a contamination with antimicrosomal antibody of the LATS-IgG containing fraction.

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ZUM KLINIKUM DER LAKTAZIDOSE

Von

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Die Laktazidose ist eine Form der metabolischen Azidose, gekennzeichnet neben übermäßiger Erhöhung des Milchsäurespiegels, durch Zunahme der Brenztraubensäurekonzentration im Blute, Erhöhung des Milchsäure/Brenztraubensäurequotienten, und häufig mit einer Verminderung des Bikarbonatgehaltes und des pH im arteriellen Blute.

Die Veränderungen der Milchsäure–Brenztraubensäure- sowie der Säure–Basenwerte im Blute wurden in 230 Fällen untersucht.

Die beobachteten Laktazidosefälle waren in der Mehrzahl mit einer bekannten Grundkrankheit verbunden. Die klinischen Erscheinungen, die erst jenseits eines Laktatspiegels von 5 mÄq/l zur Beobachtung kamen, waren von Störungen der Atmung und des Bewußtseins beherrscht. Ein im Verhältnis zu den Na- und K-Werten niedriger Cl-Plasmaspiegel gilt als Vorzeichen einer Laktazidose. Die Laktazidose schweren Grades spricht auf die Behandlung kaum an, ihre Prognose ist ungünstig.

Die Milchsäure ist ein Zwischenprodukt der anaeroben Oxydation der Kohlenhydrate. Ihre pathologische Anhäufung im Blute führt zu klinischen, mit biochemischen Veränderungen verbundenen Erscheinungen, deren Bedeutung erst in den vergangenen 10 Jahren, seit dem Erscheinen von HUCKABEES Arbeiten erkannt wurde [21—24]. In klinischer Hinsicht werden zwei Haupttypen der Laktatanhäufung unterschieden:

1) Die Laktazidose, deren Kriterien weder bezüglich der Höhe des Laktatspiegels, noch des Grades der Azidose einheitlich sind. Einzelne Autoren sprechen von Laktazidose bereits über 2 mÄq/l, andere hingegen erst im Falle einer Milchsäurekonzentration über 7 mÄq/l. Auch die pH-Werte müssen nicht vermindert sein: in TRANQUADAS Fällen war die Pufferkapazität des Organismus selbst bei einem Milchsäurespiegel über 7 mÄq/l zur Kompensation der Azidose ausreichend [39, 42, 43].

Die Laktazidose kann a) sich sekundär einem Krankheitsprozeß bekannter Ätiologie hinzugesellen, oder b) als idiopathisches Krankheitsbild in Erscheinung treten. So z. B. wurde sie bei Alkoholvergiftung, während PhenforminBehandlung, bei Leukämie, bei Gierkescher Krankheit, bei azetonämischer diabetischer Azidose sowie bei extrakorporealem Kreislauf beobachtet [2, 7, 8, 10, 13, 20, 26—29, 36, 38, 40, 44].

Die idiopathische, primäre oder spontane Form, von der neuerlich angenommen wird, daß sie eine mit latenten Kreislaufstörungen oder Präschockzuständen verbundene Stoffwechselstörung ist, die zum Tode führt, bevor

sich die Erscheinungen der Grundkrankheit entfalten konnten [15, 16, 21, 31, 32].

2) Unter Hyperlaktatämie wird eine mäßige Erhöhung des Milchsäurespiegels ohne Azidose verstanden. Ihre Ursachen sind: Muskelarbeit, Hyperventilation, Anämie, Hypoxie, Adrenalin-Einspritzung sowie Glukose- und Bikarbonat-Infusion [1, 4, 5, 12, 19, 30, 35, 36, 39, 41, 43]; das pH ist normal oder in Richtung der Alkalose verschoben.

Die mit einer pathologischen Erhöhung des Milchsäurespiegels einhergehenden Zustände lassen sich auch aufgrund des Verhaltens des Milchsäure–Brenztraubensäure-Quotienten (L/P) differenzieren. Erhöhung des L/P Quotienten, ein »lactate excess«, kommt in der Mehrheit der primären und sekundären Laktazidosen sowie bei den mit akuter Hypoxie und Adrenalinverabreichung verbundenen Laktatämien vor, während bei den übrigen Formen der Laktatämie der Quotient normal bleibt.

Die Erkennung einer Laktazidose ist nur unter Anstaltsbedingungen möglich. Wenn bei metabolischer Azidose die Bikarbonat-Konzentration niedrig, das Serum-Cl normal oder vermindert ist und sich für die Säure–Basenstörung keine Ursache finden läßt, ist stets die Möglichkeit nachzuprüfen, ob sich hinter dem scheinbaren Anionmangel eine Laktaterhöhung verbirgt, die auch für die pH-Verminderung verantwortlich ist.

Die den Gegenstand der gegenwärtigen Mitteilung bildenden Untersuchungen wurden vor 2 1/2 Jahren begonnen. Aus dem bei Bettruhe ohne Stauung entnommenen venösen Blut wurde die Milchsäure nach der modifizierten kolorimetrischen Methode von BARKER und SUMMERSON, und die Brenztraubensäure nach der Methodik von FRIEDMANN und HAUGEN bestimmt [3, 14, 25]. Insgesamt wurden 230 Kranke untersucht. Eine Laktatämie mittleren Grades (3—5 mÄq/l) ließ sich bei 44 Fällen feststellen, bei weiteren 9 Patienten war eine ausgeprägte Laktazidose (über 5 mÄq/l) vorhanden. Resuszitierte Fälle mit extremen Werten wurden bei diesen Untersuchungen nicht berücksichtigt. Die Vielfältigkeit des Bildes der Laktazidose geht auch aus den im weiteren anzuführenden Beobachtungen hervor.

Die 9 Patienten, bei denen eine ausgeprägte Laktazidose vorlag, lassen sich in 3 große Gruppen einteilen: je nachdem, ob die Laktazidose mit einem chronischen respiratorischen Prozeß, einer akuten kardiovaskulären Episode, oder einer chronischen Leberkrankheit verbunden war. Die klinischen Erscheinungen der Milchsäure-Azidose waren nicht spezifisch. Eine Hypertachypnoe verschiedenen Schweregrades ließ sich bei sämtlichen Kranken, und eine Zyanose bei den Patienten Nr. 1, 2 und 9 beobachten. Als Beschwerden wurden in jedem Falle allgemeine Schwäche, Niedergeschlagenheit und Atemnot angegeben. Mit Ausnahme des Falles Nr. 8 war das Bewußtsein in sämtlichen Fällen beeinträchtigt; gewöhnlich ließen sich Lethargie, Somnolenz, in Fällen Nr. 1, 2 und 4 sogar Stupor bzw. Koma beobachten. Die Pulsfrequenz war er-

höht, Rhythmusstörungen kamen bei Fällen Nr. 2 und 6 vor. Bei Myokardinfarkt und Pulmonalembolie war der Blutdruck herabgesetzt. Eine ausgeprägte Hypochlorämie war nur bei den Fällen Nr. 2 und 6 zu beobachten (80 bzw. 82 mÄq/l). Azotämie oder Azetonurie kam nie vor.

Die Laktazidose war stets mit einer Verschlechterung der Grundkrankheit verbunden. Auch der Brenztraubensäurespiegel zeigte in jedem Falle eine Zunahme, doch nicht in dem Grade wie der Laktatspiegel, darum blieb der L/P-Quotient stets höher als 10 (Tab. I).

Tabelle I

Fall	Alter, Geschlecht	Blutmilchsäure mÄq/l	Blutbrenztraubensäure	L/P	Diagnose
1	72 ♀	8	0,27	28,8	Emphysem, Kyphoskoliose, Cor pulmonale chr.
2	69 ♀	12,2	0,86	14,1	Embolia pulmonum
3	42 ♂	6	0,37	16	Leberzirrhose
4	62 ♂	7,1	0,56	12,5	Leberzirrhose
5	63 ♂	6,88	0,30	22,8	Leberzirrhose, Herzinfarkt
6	64 ♂	9,1	0,41	21,6	Herzinfarkt
7	69 ♂	5,3	0,27	19,5	Herzinfarkt
8	43 ♂	5,7	0,24	23,7	Herzinfarkt
9	48 ♂	5,3	0,22	24	Herzinfarkt

Eine dekompensierte metabolische Azidose war in Fällen Nr. 2 und 6 nachweisbar. Eine Verminderung der Bikarbonat-Konzentration — mit negativem BE — lag bei einem Kranken, und ein geringfügiger Basismangel bei 2 Patienten vor (Tab. II). Eine pathologische Hyperventilation — mit niedrigem pCO_2 und respiratorischer Alkalose — ließ sich bei den Kranken Nr. 3, 4, 7, 8 und 9 nachweisen und bei einem dieser (Fall Nr. 7) war die respiratorische Alkalose mit kompensierter metabolischer Azidose verbunden.

Die häufigste Abweichung bei unseren laktazidotischen Kranken war die Hypoxie. In 8 der 9 Fälle kam sie in sehr schwerem, mittelschwerem bzw. leichterem Grade zur Beobachtung.

Tabelle II

Fall	pH	Standard Bicarb. mÄq/l	Base Excess mÄq/l	pCO_2 mmHg	pO_2 mmHg
1	7,375	23,1	—0,9	42	61,5
2	7,24	17,6	—9,2	47	37
3	7,45	21,2	—3,8	31,5	86
4	7,44	22,6	—1,6	31,5	90
5	7,38	22,1	—2,1	38	68
6	7,21	12	—17	32	70
7	7,41	18,6	—7,4	22	49
8	7,44	21,1	—3,8	25,5	85
9	7,43	22,6	—1,9	28,5	78

Aufgrund unserer Beobachtungen und der Angaben des Schrifttums ist bei Laktazidose die pathologische Hyperventilation oder die Hypoxie selbst beim Fehlen einer systematischen Azidose stets nachweisbar [6, 37]. Die Ursache der bei passiver oder pathologischer Hyperventilation erfolgenden Milchsäure-Anhäufung ist unbekannt, es läßt sich aber annehmen, daß die Aktivität der Pyruvat-Karboxylase durch die intrazelluläre Hypokapnie beeinträchtigt wird und darum die Reduktion der Brenztraubensäure zu Milchsäure nur auf anaerobem Wege möglich ist. Eine andere Erklärung wäre, daß die Leber bei intrazellulärer Alkalose infolge der verminderten Perfusion weniger Laktat aufnimmt.

Bei sämtlichen Laktazidoseformen fällt der Leber eine zentrale Rolle zu [9, 17]. Die Aufnahmefähigkeit der Leber für Milchsäure ist aber weit größer als die von den extrahepatischen milchsäureproduzierenden Geweben erzeugten Mengen, deshalb kann es sich bei Laktazidose um keine einfache Überproduktion an Milchsäure handeln, sondern es muß eine mangelhafte Verwertung infolge der krankhaften Vorgänge in der Leber oder der Durchblutungsstörungen im Splanchnicusgebiet vorliegen.

Die in Begleitung einer Hypoxie kardiovaskulären oder respiratorischen Ursprungs auftretende Laktazidose ist von prognostischer Bedeutung: nach PERETZ bleiben bei einer Milchsäurekonzentration unter 4,3 mÄq/l 100%, zwischen 4,4—8 mÄq/l 33%, über 8,8 mÄq/ jedoch nur 15% der Kranken am Leben [33].

Auch im gegenwärtigen Material wurde nur eine Laktazidose mäßigen Grades (unter 6 mÄq/l) überlebt bzw. von Besserung gefolgt. Die Kranken Nr. 7 und 9 starben später an einer anderen Komplikation der Grundkrankheit.

Eine Laktazidose in Verbindung mit Versagen des Kreislaufes oder der Respiration kam nur bei akuten Episoden bzw. akuten Rückfällen zur Beobachtung, z. B. bei frischen Myokardinfarkten, Pulmonalembolien oder bei

Exazerbationen des chronischen Cor pulmonale. Bei chronischen Hypoxiezuständen ließ sich keine bedeutende Laktazidose nachweisen.

Die Laktazidose ist eigentlich eine Stoffwechselstörung, die intrazellulär in Gang gesetzt wird; so ist sie keiner quantitativen Bestimmung zugänglich, auch durch die Therapie werden nur ihre sich auf den Extrazellulärraum projizierenden Verschiebungen erfaßt.

Die Grundlagen der Behandlung sind:

1) Elimination des Laktat-Überschusses mittels Wiederherstellung des peripheren Kreislaufs,

2) Korrektion der Azidose mit alkalisierenden Lösungen und

3) Bindung des zirkulierenden Laktats auf pharmakologischem Wege, d. h. mittels Methylenblau, — oder seine Beseitigung auf mechanischem Wege, mittels Dialyse.

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STIMULATION OF THE LYMPHATIC ORGANS IN VIVO

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Continued administration of heterogenic blood (HB) and of phytohaemagglutinin (PHA) by the intravenous route to rabbits resulted in typical changes of all, peripheral and central, lymphatic organs, characterized by a shift in the size distribution of the lymphocyte population with a prevalence of large lymphocytes, formation of blast cells and appearance of dividing forms. This response may be regarded as a counterpart in vivo to the reactions exhibited by lymphocyte cultures to mitogenic stimuli, and corresponds in its cytologic features to some pathologic lymph node changes by humans characterized by the prevalence of large lymphocytes and the formation of blast cells, with individual specific alterations. On these grounds it is suggested that the cytological changes arising in response to lymphocyte stimulation represent an adequate reaction of lymphocytes.

In addition to these changes involving the entire lymphatic system there may be particular, organ-related responses which are probably specific to the individual organs. Reactions of this kind, such as extramedullary haemopoiesis in the spleen and thymus or mastocyte reaction in the thymus, may possibly involve a further proliferative capacity of blast cells.

The discovery of NOWELL [19] that phytohaemagglutinin (PHA) induces in lymph cell cultures the transformation of small lymphocytes into large, active cells capable of cell division has opened up new aspects in research relating to the lymphatic system. It has been established that the small lymphocytes represent a quiescent cell form of the lymphatic system, capable of further development rather than the end stage of cell transformation [29]. In the last years, the literature on the subject has become too vast to be kept in evidence. Here we only refer to the monograph of LING [12] and to the papers of ASTALDI et al. [2, 3] and of LOZZIO [13].

The effects of PHA in vivo have failed to attract general interest. Apart from clinical trials of questionable outcome in aplastic anaemia [7, 10, 11], the effects of PHA on the lymphatic organs have received limited attention in the literature [5, 8, 18, 21, 24, 25, etc.].

The aim of the present study has been to clarify the effect of PHA on the living organism, particularly on the lymphatic organs, by comparing its effects with those of other antigens, on the one hand, and with its own effects in vitro, on the other. In addition, the cytological response to the experimental stimulation of the lymphatic organs has been compared with the cytological features of diseases of the lymphatic organs in humans.

Material and methods

Rabbits weighing approximately 2.0 kg, of random sex and breed, were used. They were kept on dry synthetic food for four weeks before and during the period of study. Several groups were set up, each including ten test animals and two controls.

The antigen used was heterogenic blood (HB), Rh negative human group O blood being injected in doses of 1.0 ml/kg daily into the marginal ear vein for 21 days. The animals were sacrificed and processed histologically and cytologically, on the 21st day. The organs and tissues studied included the spleen, lymph nodes, thymus, appendix, bone marrow and liver. Individual animals of each group were occasionally sacrificed earlier or later than the 21st day, according to the purpose of the experiment.

PHA (Difco P) was injected intravenously in doses of 0.1 mg/kg daily, likewise for 21 days. Processing was the same as in the HB-treated groups.

The HB-treated animals appeared to be in poor health and failed to increase in weight during treatment, there were, however, only occasional deaths. In the groups allowed to survive, regeneration was rapid and control body weight was regained in two or three weeks. The PHA-treated animals had a normal appearance with normally ascending weight curves during the entire period of treatment. There were a few occasional deaths, mostly unrelated to the treatment.

Results

Before describing the abnormalities of the lymphatic organs in detail, we have to outline the main abnormal cytologic features of the peripheral blood. This question has been dealt with in detail in an earlier study [23]. The differential blood count was checked twice weekly during the entire period of study, the last test being performed on the morning of the animals' sacrifice.

Apart from a transitory leukocytosis the blood counts revealed, as the most characteristic feature, a striking increase in the number of large lymphocytes attaining 80% or even more of the total lymphoid cell population by the end of the experiment. The population of large lymphocytes contained numerous cells of bizarre configuration similar to glandular fever cells but there were also clearly recognizable blast cells and dividing forms. In general the cytology of peripheral blood was reminiscent of that associated with infectious mononucleosis, in the HB- and PHA-treated animals alike, with the only difference that in the PHA-group the leukocytosis was of lesser degree.

The gross appearance was characterized by an enlargement of the lymphatic organs which, however, revealed different features according to the stimulus applied. In the HB-treated animals the spleen was considerably enlarged, weighing in general 5.0 g, in some cases even more than 10.0 g, the normal range in the control animals being between 0.5 and 1.0 g. The mesenteric lymph nodes formed huge conglomerates weighing several grams. The thymus was moderately enlarged in some animals, completely atrophied in some others.

In the PHA-treated animals the spleen was smaller, weighing between 2.0 and 3.0 g. The mesenteric lymph nodes were similar in size to those of the HB-treated series. The thymus was considerably larger than in the other group and there was scarcely any atrophied organ in this series.

The most important cytological changes concerned the proportion between the small and the large lymphocytes. In normal untreated animals the distribution of small and large cells in the lymphocyte population corresponds to 70 : 10. The HB-treated animals examined at the end of the first week still showed the same distribution, while at the end of the second week the proportion of large lymphocytes was 30% and continued to rise, attaining by the end of the experiment 50 to 60%. In addition, numerous blast cells were seen. In respect of morphology, the transition from the small lymphocytes, through the large ones, to the blast cells was unbroken.

It is to be mentioned that the term "large" lymphocyte is used in a collective sense to denote all lymphocytes once having moved in any direction from the quiescent state represented, according to the general view, by the small lymphocytes. The use of the term "medium-sized" lymphocytes is being refrained from. It must be emphasized that from the end of the second week onward numerous cells of different sizes were noted among the large lymphocytes in the spleen of the HB-treated but also of the PHA-treated animals. There was a practically unbroken transition from the small lymphocytes to the blast cells, in other words, the cytological response in vivo was of the same pattern as in PHA-treated lymphocyte cultures.

The spleen of HB-treated animals exhibited a marked plasmacellular reaction, the plasma cells in various stages of maturity accounting for 15 to 20% of the entire population of nucleated cells by the end of the experiment. This was accompanied by an intensive myelopoiesis in the spleen; the myeloid cell population was prevalently formed by young promyelocytes and myelocytes, apart from numerous normoblasts.

In the spleen of the PHA-treated animals the changes were of the same character as in the former group with the only difference that the plasmacellular reaction as well as myelopoiesis appeared of lesser intensity. However, in respect of the shift in the ratio of small to large lymphocytes, the response in the PHA-group differed neither quantitatively nor qualitatively from that exhibited by the HB-group, the predominance of large lymphocytes and the presence of numerous blast cells by the end of the experiment being typical for both groups (Figs 1, 2).

Nothing could be more illustrative of the profound cytological changes taking place in the spleen than the fact that not only has the organ multiplied its original size, its cell population being many times that of the original number, but it has been completely reorganized in respect of its cellular substrate, the myeloid elements and plasma cells, normally few in number, accounting for approximately one third of the entire nucleated cell population.

As mentioned before, the mesenteric lymph nodes were significantly enlarged in both groups to nearly the same extent. No significant difference was found in the cytological pattern either. The essential feature was a shift

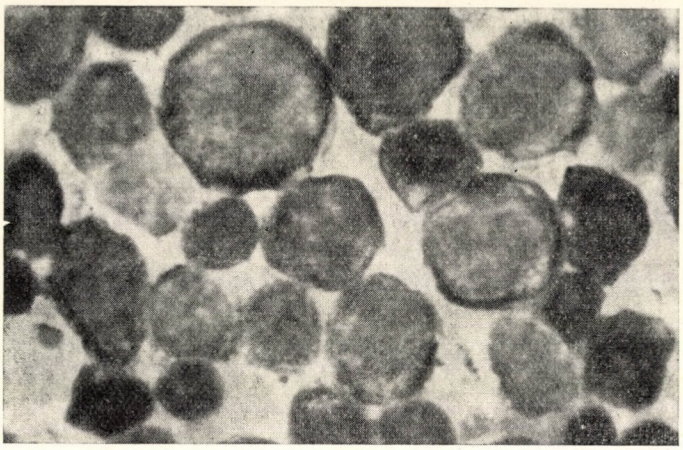

Fig. 1. Spleen of HB-stimulated animal. 22nd day. May—Grünwald—Giemsa stain, ×800

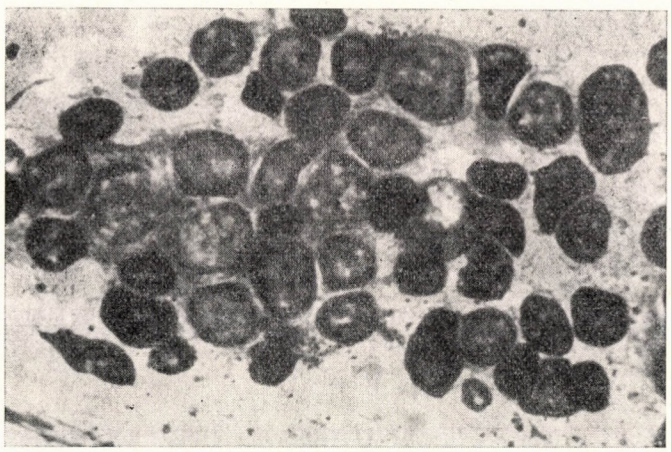

Fig. 2. Spleen of PHA-stimulated animal. 22nd day. May—Grünwald—Giemsa stain, ×800

in the ratio of small to large lymphocytes; by the end of treatment the cell population was prevalently formed by large lymphocytes. In addition, there were numerous blast cells, amounting in the PHA-group to 5% and in the HB-group to 6.2%. The cytoplasm of these cells exhibited a marked pyroninophilia upon methylgreen pyronine staining. Here again, an unbroken transition from small lymphocytes (quiescent form) to large lymphocytes and blast cells was demonstrable. In consideration of the additional finding of numerous dividing forms, the cytological pattern must be regarded as strongly reminiscent of that exhibited by PHA-stimulated lymphocyte cultures (Figs 3, 4). As regards the other cell elements, there was a moderate increase in the number of plasma cells attaining 1 to 2% and occasional normoblasts were found. The number and activity of macrophages was increased, there were numerous

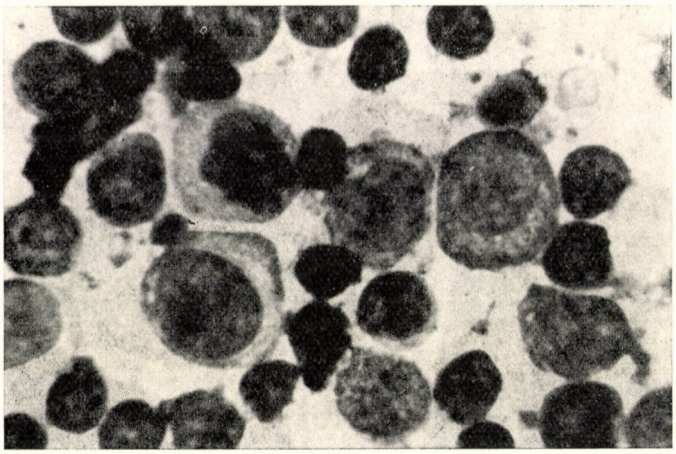

Fig. 3. Lymph node of HB-stimulated animal. 22nd day. May—Grünwald—Giemsa stain, ×800

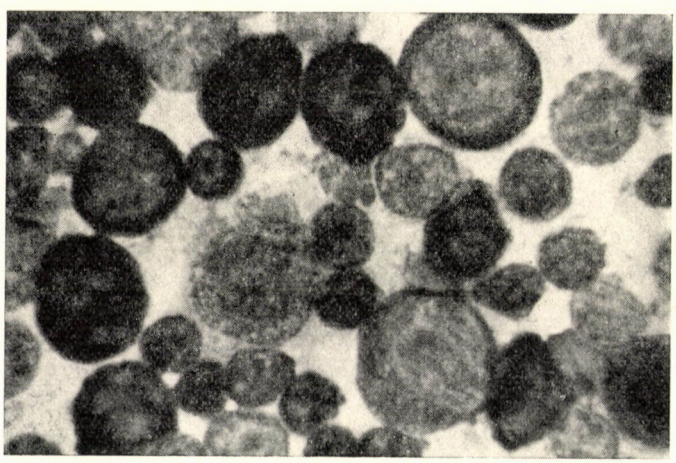

Fig. 4. Lymph node of PHA-stimulated animal. 22nd day. May—Grünwald—Giemsa stain, ×800

cells with phagocyted erythrocytes and leukocytes. It could be established from the pseudoeosinophilic granulations that these granulocytes were autogenous. The reticular cells were also increased in number. The number of the non-lymphocytes none the less failed to attain 5% of the cell population.

On comparison of the lymph nodes of HB-treated with those of PAH-treated animals, the two types of stimulation were found to affect the lymph nodes in the same manner.

It seemed of interest to confront this evidence with the findings derived from lymph node biopsy in human diseases. A few illustrative cases are shown in Figs 5 to 11.

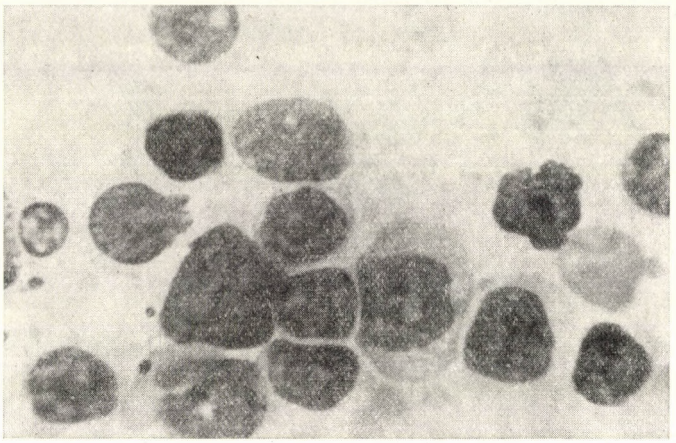

Fig. 5. Mrs. S. J. Infective hepatitis. Cervical lymph node biopsy. May—Grünwald—Giemsa stain, ×800

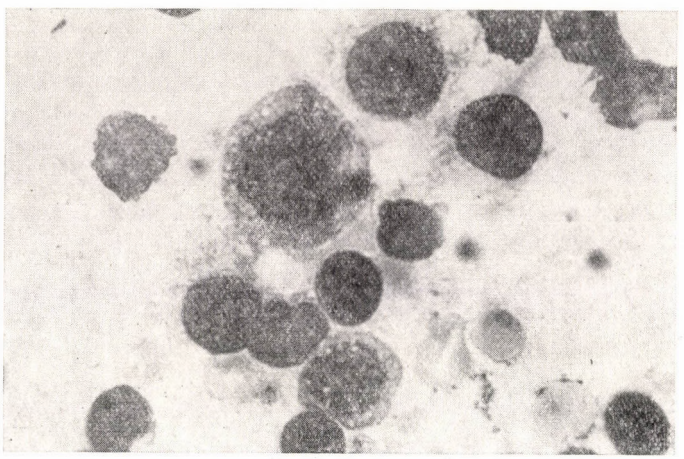

Fig. 6. V. J. Non-specific lymphadenitis. Cervical lymph node biopsy. May—Grünwald—Giemsa stain, ×800

The material obtained from human cases shown in Figs 5 to 11 reveals a striking resemblance of the changes to those of the experimentally stimulated lymph nodes. Here too, aside from the possible specific alterations, there is a conspicuous predominance of large lymphocytes and blast cells. These changes which are analogous with those exhibited by stimulated lymphocyte cultures, seem to accompany the specific lesions in human lymph nodes and even precede them in time.

As regards the central lymphatic organs it is the thymus which we have to discuss first. The part played by the thymocytes is still a subject of controversy in the literature. It is claimed, for instance, that lymphopoiesis of the

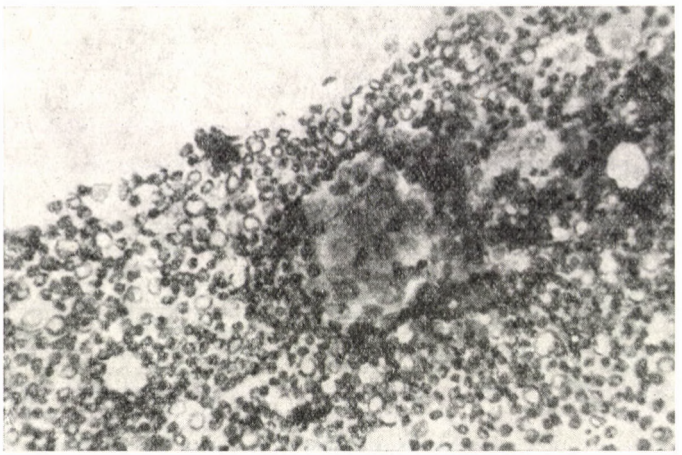

Fig. 7. Mrs. Sz. G. Tuberculous lymphadenitis. Cervical lymph node biopsy. Tubercle. ×350

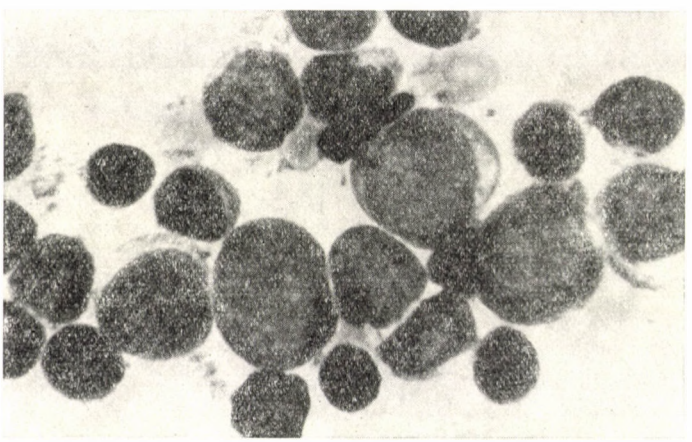

Fig. 8. Same as in Fig. 7. A portion, farther away from the tubercle. ×800

thymus is independent of any antigenic stimulation [17] or that the thymocytes are not directly involved in any reaction directed against foreign antigens [16]. According to METCALF [15], the thymocytes are antigen-unresponsive. Correspondingly, plasma cells are practically absent from the thymus. On the other hand, various authors including MARSHALL and WHITE [14] noted a regular immune reaction in laboratory animals with the appearance of germinative centers and numerous plasma cells in response to a direct intrathymic injection of the antigen.

In the present study, HB as well as PHA stimulation was found to induce an enlargement of the thymus which was particularly marked in the PHA group. The essential microscopic features were similar to those discussed

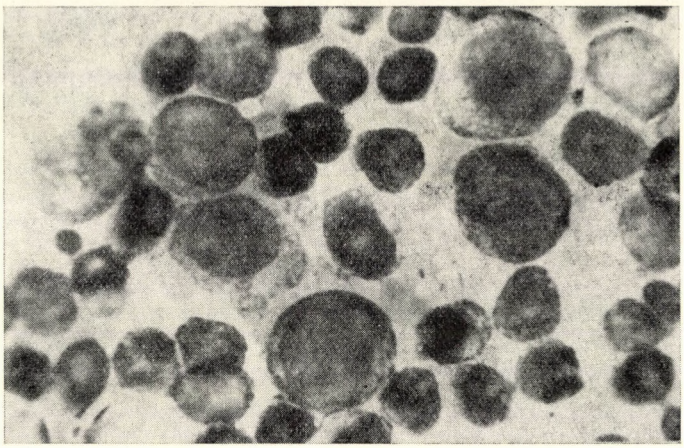

Fig. 9. Z. R. Toxoplasmosis. Cervical lymph node. ×800

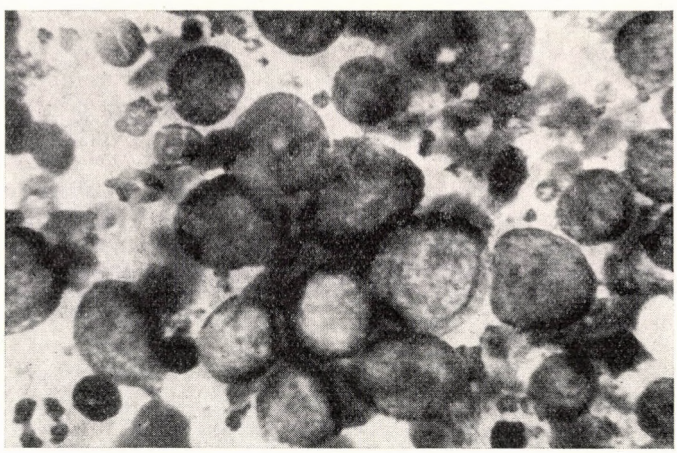

Fig. 10. P. J. Lymphogranulomatosis. Axillary lymph node. ×800

earlier in respect of the other lymphatic organs, namely a prevalence of large lymphocytes and blast-cell transformation. This reaction seems to take a similar course in the thymus as in the peripheral lymphatic organs (Figs 12, 13). In addition to the changes described above, sporadic plasma cells were also detectable. The appearance of young myeloid elements was fairly common which seems to support the view of those regarding the thymus as a haemopoietic organ [28, 20]. A marked mastocyte reaction, interpreted by TÖRŐ [27] and by CSABA [4] as a nonspecific response of the thymus, was also demonstrable. In this context we have to mention the finding, though without the intention of drawing any far-reaching conclusion from it,

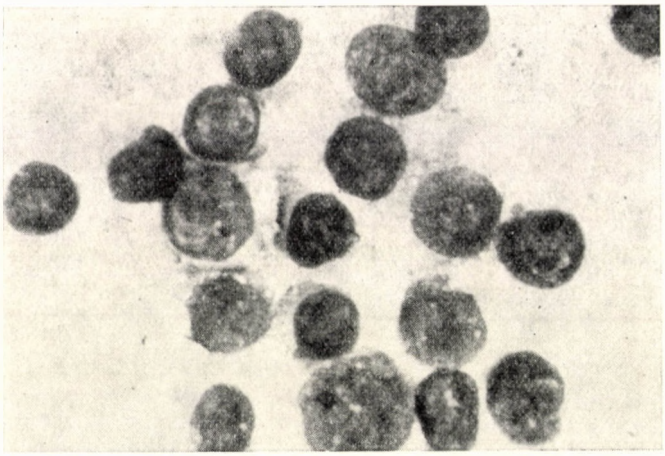

Fig. 11. Mrs. N. C. Breast cancer. Axillary lymph node free from metastases. ×800

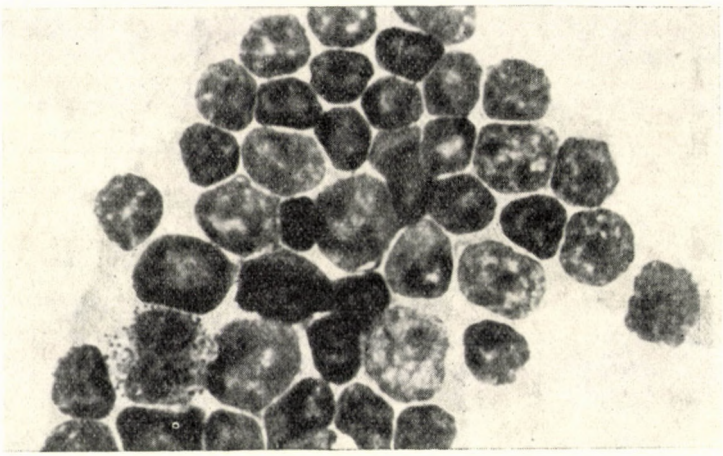

Fig. 12. HB-stimulated rabbit. Thymus. May—Grünwald—Giemsa stain, ×800

that the peripheral basophile count was 6 to 9% in the PHA-group and 4 to 6% in the HB-group at the end of treatment.

The other central lymphatic organ is the bursa of Fabricius in birds. Its counterpart in mammals is still unidentified but it must be presumably sought in the lymphoepithelial structures scattered along the entire gastrointestinal canal, from Waldeyer's ring down to almost the rectum, provided the function of the bursa should be confined in mammals too to any single organ instead of being taken over by the entire system.

TURBA [26] called attention as early as 1921 to the presence of numerous lymph follicles in the vermiform appendix and in the adjoining sacculus rotun-

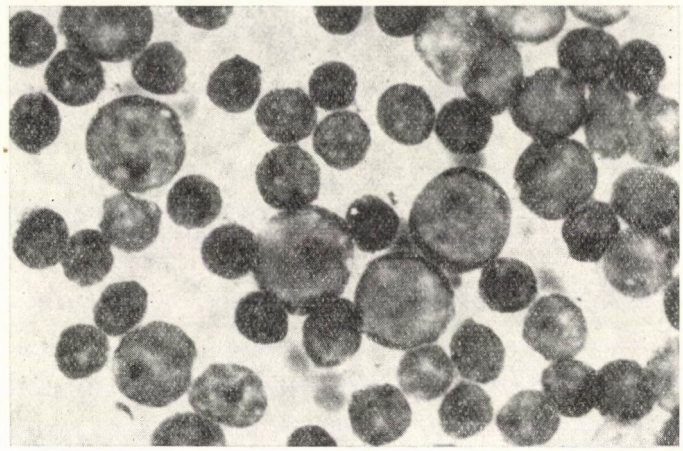

Fig. 13. PHA-stimulated rabbit. May—Grünwald—Giemsa stain, ×800

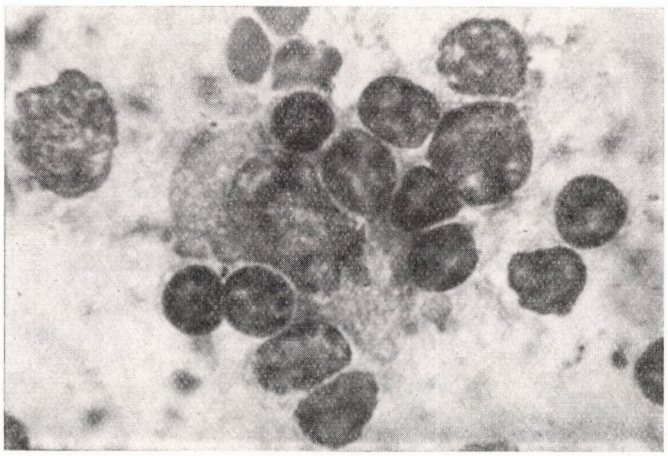

Fig. 14. HB-stimulated rabbit. Appendix. ×800

dus in rabbits. In the view of FARKAS [6] it would be tempting to regard the vermiform appendix as a central reactive lymphatic organ of the same biological properties as the tonsils. In fact, it has been shown by ARCHER et al. [1] that not only does the vermiform appendix of rabbits closely resemble the bursa of Fabricius in morphological and embryological respects but it also plays the same part in the immune mechanisms and now it is generally accepted that the vermiform appendix in rabbits corresponds to the bursa of Fabricius. In view of this fact the cytology of this organ has also been examined in the present study.

Macroscopically, the vermiform appendix in rabbits is an 8 to 10 cm long, 1.5 to 2 cm wide structure lined with a thick, velvety mucous membrane.

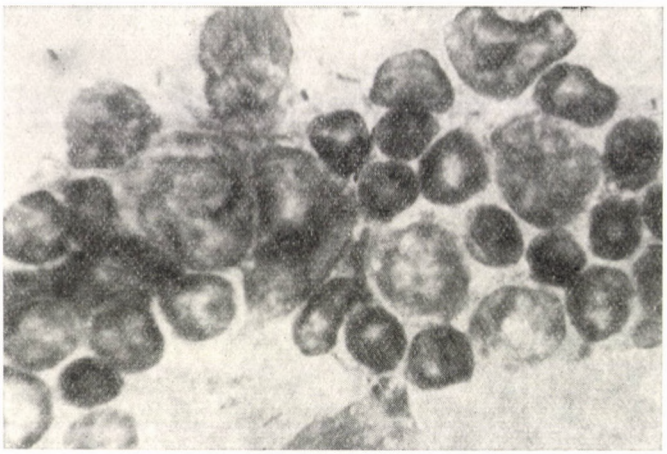

Fig. 15. PHA-stimulated rabbit. Appendix. ×800

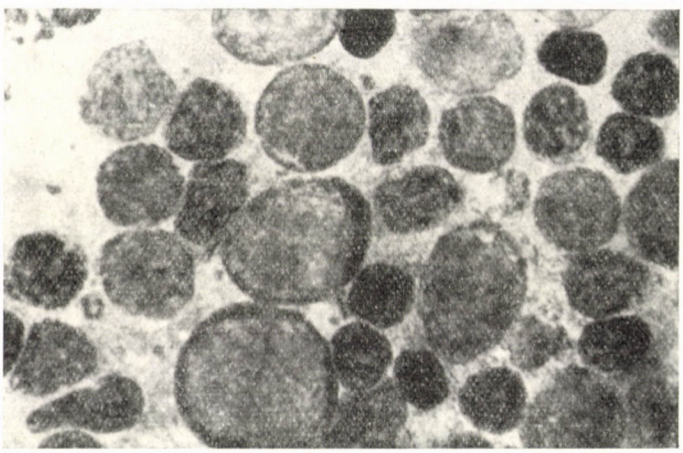

Fig. 16. U. L. aged 12. Tonsil. Chronic tonsillitis ×800

The cytological pattern largely corresponds to that of other lymphatic organs, with the only possible difference that here the number of large lymphocytes is higher. There is a fair number of giant cells of epitheloid type to which the surrounding lymphocytes seem to adhere. Changes of similar kind were found sporadically in the thymus, too, and are common in smears of human tonsils.

The appendix of the stimulated animals exhibited practically the same changes as did the other lymphatic organs, i.e. a shift of the lymphocyte population toward the large lymphocytes. The ratio of small to large lymphocytes was significantly reduced. In addition to the predominating large lymphocytes there were numerous blast cells. No plasmacellular reaction was noted. Only sporadic plasma cells were detectable (Figs 14, 15).

We have yet to refer briefly to the tonsils in human which in their structure as well as in their evolution and regression are reminiscent of the thymus and may be regarded, if not as the sole representative, at any rate as a member in the system of lymphoid organs and structures which are considered the equivalents of the bursa of Fabricius in humans.

Smears of tonsils removed for chronic inflammation were examined. The microscopic finding was marked by numerous large follicles and from the cytological features it clearly emerged that the tonsillar lymphocytes respond

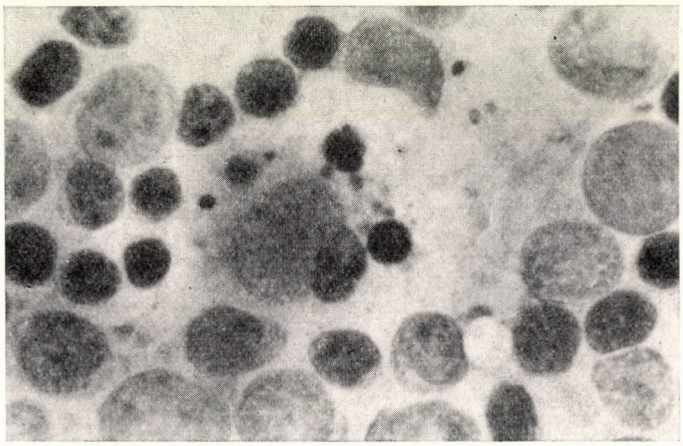

Fig. 17. M. É., aged 27. Tonsil. Chronic tonsillitis ×800

to stimuli (in this case probably bacterial antigens) in the same manner as does any other lymphoid tissue. The overwhelming majority of the lymphocyte population was made up of large lymphocytes; dividing cells and particularly blast cells were present in large numbers. Plasma cells were by no means rare, even plasmacellular syncytia and islets were seen. It was interesting to note peculiar connections between the giant cells and the lymphocytes; the latter cells were found to adhere firmly to the giant cells and even to intrude into their cytoplasm where they were for the greatest part destroyed (Figs 16, 17).

Discussion

It was observed that in short-lived cell-cultures the small lymphocytes start growing, dividing and undergo transformation into blast cells on exposure to PHA, antigens, foreign leukocytes and even to physical, e.g. ultrasonic, stimuli. This has prompted us to examine whether the lymphocytes of the living organism would respond to similar stimuli in a similar manner. If so, we proposed to examine whether this reaction involved the cells of the

entire lymphatic system, consideration being given to the cells of the central lymphatic organs which distinctly differ in their types of response from the peripheral organs.

The present results showed that stimulation with PHA or HB elicits an identical pattern of response of the entire lymphatic system including the central organs of lymphoepithelial structure such as the thymus, the vermiform appendix and human tonsils. The essential features of the reaction include a significant enlargement of the lymphatic organs and a shift in the proportion of the lymphocyte population towards a prevalence of large lymphocytes, numerous blast cells and dividing forms. This response basically corresponds to that exhibited by stimulated lymphocyte cultures.

On confrontation of this material with the cytological findings characteristic of human lymphoglandular diseases we find their basic features strikingly identical. Prevalence of large lymphocytes and blast-cell transformation were invariably demonstrated side by side with individual specific changes. This response not only occurs in diseases caused by lymphotrophic viruses where this kind of reaction would be typical [9], but practically in every disease involving the lymphatic system.

In view of the facts that (1) lymphocytes in cultures are equally responsive to specific and nonspecific mitogens as well as to simple chemicals or to physical factors; (2) the lymphatic system is likewise responsive to these stimuli in vivo and (3) human diseases involving the lymphatic system exhibit similar cytological features, it is safe to assume that the changes arising in response to the stimuli, i.e. formation of large lymphocytes, blast-cell transformation, mitosis, actually represent adequate reactions of the lymphocytes to any stimulus that may affect them.

While the type of response dealt with in the foregoing involves the entire lymphatic system, there are certain organ-related additional reactions depending on the character of the individual organ, such as extramedullary haematopoiesis in the spleen and thymus, plasmacellular metamorphosis in the spleen and tonsils, generation of mastocytes in the thymus. A further transformation of blast cells resulting in various other cell groups may well provide the cytological basis for reactions of this kind.

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CLINICAL AND HISTOPATHOLOGICAL STUDIES IN HUMAN RENAL DISEASE

I. SOME ASPECTS OF THE PATHOGENESIS AND AGE-RELATIONSHIP OF GLOMERULONEPHRITIS

By

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Evidence indicating that the immune deposits of granular type formed on the glomerular basement membrane are connected in the majority of cases with infection presumably of streptococcal origin, in contrast to the deposits of linear character revealing no relationships of this kind, has been substantiated by the present study on the grounds of the morphological features and the clinical course of the process. In the younger age groups (10—29) immune deposits of the linear type were significantly more frequent than in more advanced age, a finding which seems to suggest that glomerulonephritis occurring at this time of life is prevalently of nephrotoxic origin. On the other hand, over 40 years of age the incidence of granular type immune deposits was significantly higher than that of the linear ones and over 50 years of age the gammaglobulin and complement formed on the glomerular basement membrane were exclusively granular in type. These morphological relationships suggest that glomerulonephritis occurring later in life is mostly consecutive to upper respiratory tract infection presumably of streptococcal origin. As far as membraneous glomerulonephritis is concerned morphological signs of chronicity were found in a larger number of cases with granular type deposits which suggests that nephritis after respiratory tract infections presumably of streptococcal origin is more liable to chronic transformation than is nephrotoxic nephritis.

The genesis of human glomerulonephritis cannot be reduced to one pathogenetic factor. More factors may have a role. The aim of the present study has been to examine whether and how far the morphological features of renal biopsy material can be related to the primary factor initiating the process.

There has been mounting evidence in recent years that the pathogenesis of human diffuse glomerulonephritis involves immunological factors. The increasing use of renal biopsy has improved the understanding of renal disease by allowing to study its clinical manifestations in the context of morphological features, and to follow up the course of the process in its earlier phases instead of having to rely on postmortem findings marking the terminal stage. Immunohistological and electronmicroscopical investigations also helped to clarify the pathogenesis of the individual types of nephritis.

Experimental glomerulonephritis provides an important source of information on the pathogenesis of human glomerulonephritis by sharing many of its morphological and clinical features. The experimental models of this kind may thus give a closer insight into the evolution of human glomerulonephritis.

One of the methods for inducing in the laboratory animal morphological and clinical manifestations similar to those of human glomerulonephritis is the experimental antigen-antibody complex-nephritis when foreign protein such as horse serum administered in massive single doses or in multiple small doses [4, 13] results in glomerulonephritis which is characterized by gamma-globulin and complement deposits of granular type on the basement membrane, and its ultramicroscopical pattern by an electron-dense material on the epithelial site of the basement membrane. These features are typical of experimental antigen-antibody complex-nephritis as well as in the majority of cases of human glomerulonephritis developing after streptococcal infection. Similar changes have been described in SLE-nephritis as well [2, 5, 10, 11].

The other type of experimental nephritis which has similar clinical and morphological features as human nephritis is Masugi's nephritis. The immunohistological features of this type of nephritis of nephrotoxic pathomechanism is marked by a linear type IgG and C' localized on the glomerular basement membrane [1, 3, 16, 17], whereas it is on its endothelial site electronmicroscopically that the electron-dense deposit appears. Since in this type of nephritis the circulating antiglomerular antibody reacts with the glomerular antigen, it is actually the antiglomerular antibody which accounts for the changes. Certain types of human glomerulonephritis display morphological features of similar character, suggesting that here an antiglomerular antibody is responsible for the process [7].

The aim of the present study has been to ascertain on the basis of our renal biopsy material whether and how far the clinical history and the morphological features allow to draw any valid inference on the pathogenesis in a given case of nephritis. The age-relationship of the morphological features of nephritis has also been studied.

Material and method

Needle biopsy of the kidney was performed with the aid of X-ray television screen by the method of KARK and BUENGER [6], using a Franklin—Vim—Silverman needle.

The renal tissue thus removed was fixed immediately in 4 °C ethanol and embedded by the method of SAINTE-MARIE [14], for light microscopy sections of 4 μ were prepared and stained with haematoxylin-eosin, Jones methenamine silver, PAS, Hart and Congo red dyes. For immunofluorescence studies the sections were incubated with antihuman gamma globulin, complement, fibrin and gamma-M (C. Hyland Travenol International GMbH) conjugated to fluorescein-isothiocyanate (FITC). Specificity of the process was checked by means of inhibition.

The light-miscroscopic and fluorescence-microscopic studies were performed with an OPTON microscope, using a HBO 200 W Osram lamp. The photographs were made with an automatic camera attached to the microscope.

For electronmicroscopy, the biopsy specimens were fixed in osmium after short fixation in cold glutaraldehyde and embedded in Araldit. Ultra-thin sections were prepared by a Reichert ultramicrotome, contrasted with lead citrate and examined partly with a Philips, partly with a Hitachi-Hu-10 electronmicroscope.

Results

The present study concerns 180 cases of needle biopsy. The oldest individual was 60, the youngest 11 years old. Average age was highest in the group of nephropathy associated with scleroderma (Table I).

Table I

Histological diagnosis	Clinical diagnosis	No. of cases	Sex		Age (years)		
			female	male	average	youngest	oldest
Membraneous glomerulonephritis	Nephrotic syndrome Acute nephritis Proteinuria Orthostatic albuminuria Haematuria Chronic nephritis Pyelonephritis	120	64	56	29.2	11	60
Membranoproliferative glomerulonephritis	Nephrotic syndrome Acute nephritis Proteinuria Chronic nephritis	12	7	5	24.2	15	30
Subacute glomerulonephritis	Subacute glomerulonephritis	2	—	2	32.5	23	42
Chronic glomerulonephritis	Nephrotic syndrome Acute nephritis Chronic nephritis	14	4	10	31.6	17	52
Focal glomerulonephritis	Nephrotic syndrome	7	6	1	27.7	14	36
Lupus nephritis	SLE	6	5	1	37.3	30	46
Scleroderma with renal involvement	Scleroderma	4	3	1	38.2	26	50
Amyloid nephrosis	Amyloid nephrosis Nephrotic syndrome	4	2	2	27	21	34
Vascular nephropathy	Chronic nephritis Hypertensive disease Proteinuria	11	6	5	32	16	48

90.8% of the 548 nephritis patients making up the material of the Second Department of Medicine in the last four years were under 50 years of age, 5% between 50 and 60 and 4.2% over 60. The reason why renal biopsy was scarcely ever carried out beyond the sixth decade was because most of these patients were uraemic.

A total of 120 cases of membraneous glomerulonephritis have been analyzed in order to gain closer insight into the pathogenesis of glomerulo-

nephritis. Histological and immunohistological studies were performed in every case and additional electron microscopical investigations in 49 cases.

Light microscopical analysis revealed a diffuse thickening of the glomerular basement membrane in all of the cases and it had a similar morphological appearance regardless of age. However, in 9 cases vascular changes in the form of lamellar hyperelastosis of the interlobular artery were also

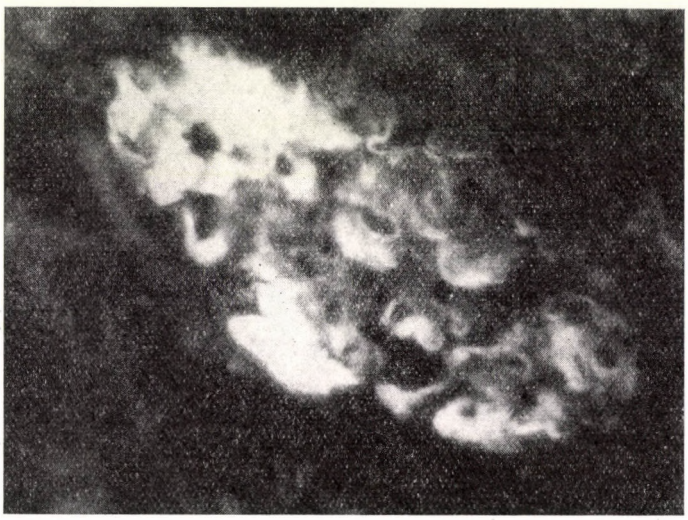

Fig. 1. Gamma-globulin of granular type on glomerular basement membrane in membraneous glomerulonephritis. (The section has been incubated with anti-human gamma-globulin conjugated to FITC)

demonstrable. In the cases with vascular involvement the average age was 41 years. There was no correlation between blood pressure and these changes. However, the history of several patients included earlier treatment for arterial hypertension or acute nephritis.

In 28 of the 120 cases of membraneous glomerulonephritis initial signs of transformation into the chronic stage were noted, i.e. glomerular hyalinization and adherence of the loops to Bowman's capsule, changes confined to one or two glomeruli, as also a simultaneous diffuse thickening of the basement membrane in the other glomerular areas. Mean age in these cases was 33.6 years.

On the evidence of immunohistological studies, in 58 cases the gamma-globulin and complement deposits on the glomerular basement membrane were of granular type (Fig. 1). In a few cases, the basement membrane of the tubules also revealed fluorescence, in contrast to the tubular epithelial cells and the vessels which displayed no fluorescence. Specificity was confirmed by

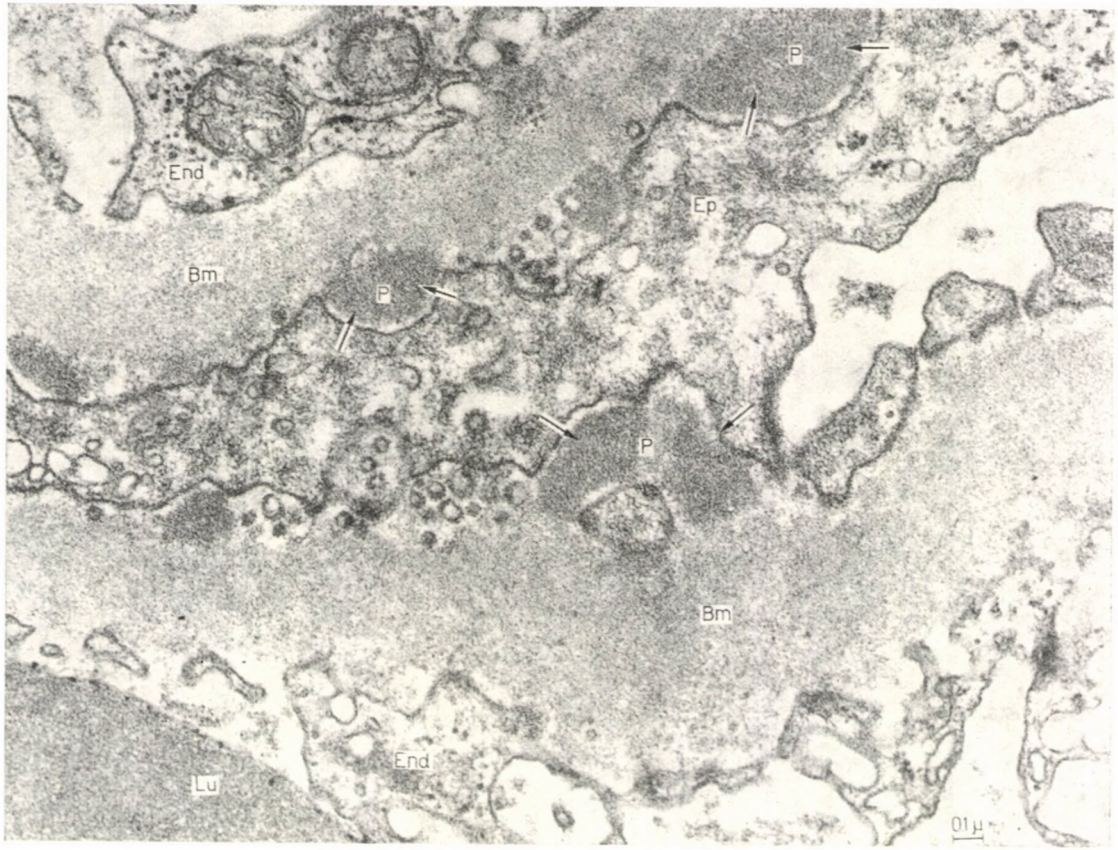

Fig. 2. Electronmicroscopical findings: the glomerular basement membrane forms multiple spinelike projections bulging toward the epithelial site. At the sites of these projections there is an electron-dense precipitate on the basement membrane (×56,000) Abbreviations: Bm = Basement membrane. Ep = Epithelial cell. End = Endothelial cell. Lu = Lumen. P = Precipitate

the inhibition test which failed to reveal fluorescence on the glomerular basement membrane in any instance. On the evidence of electron microscopy, the electron-dense material was found to appear on the epithelial surface of the basement membrane (Fig. 2). The epithelial cells revealed occasional cytoplasmic lipid droplets and microvilli.

In 54 cases, the gamma-globulin and complement deposits on the glomerular basement membrane were linear type (Fig. 3). Here too, while the

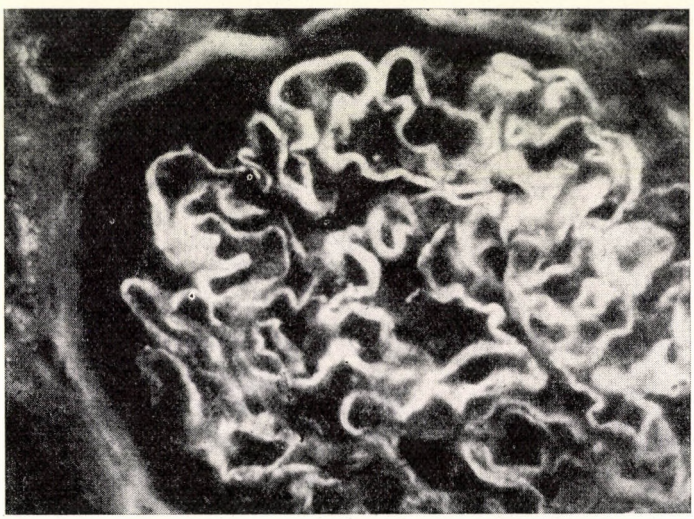

Fig. 3. Membraneous glomerulonephritis. Immunohistology revealed gamma-globulin of linear type on glomerular basement membrane. (The section has been incubated with anti-human gamma-globulin conjugated to FITC)

tubular basement membrane revealed fluorescence, the tubular epithelial cells and vessels failed to do so. No fluorescence was demonstrable by the inhibition test, as a sign of the specificity of the process. The electron-dense material was found on the endothelial surface of the basement membrane (Fig. 4). The basement membrane was thickened and an occasional fusion of the foot processes was noted.

In a few cases of membraneous glomerulonephritis, sparse deposits of fibrin of granular type were also demonstrable, partly on the basement membrane, partly in the mesangium. In addition, the glomerular basement membrane revealed IgM too in a few cases.

In eight cases, IgG and C' appeared in a focal form, too sparse to be identified as linear or granular type.

As mentioned earlier, the appearance of granular type immune deposits on the glomerular basement membrane is regarded by numerous authors as being typical of glomerulonephritis following streptococcal infection,

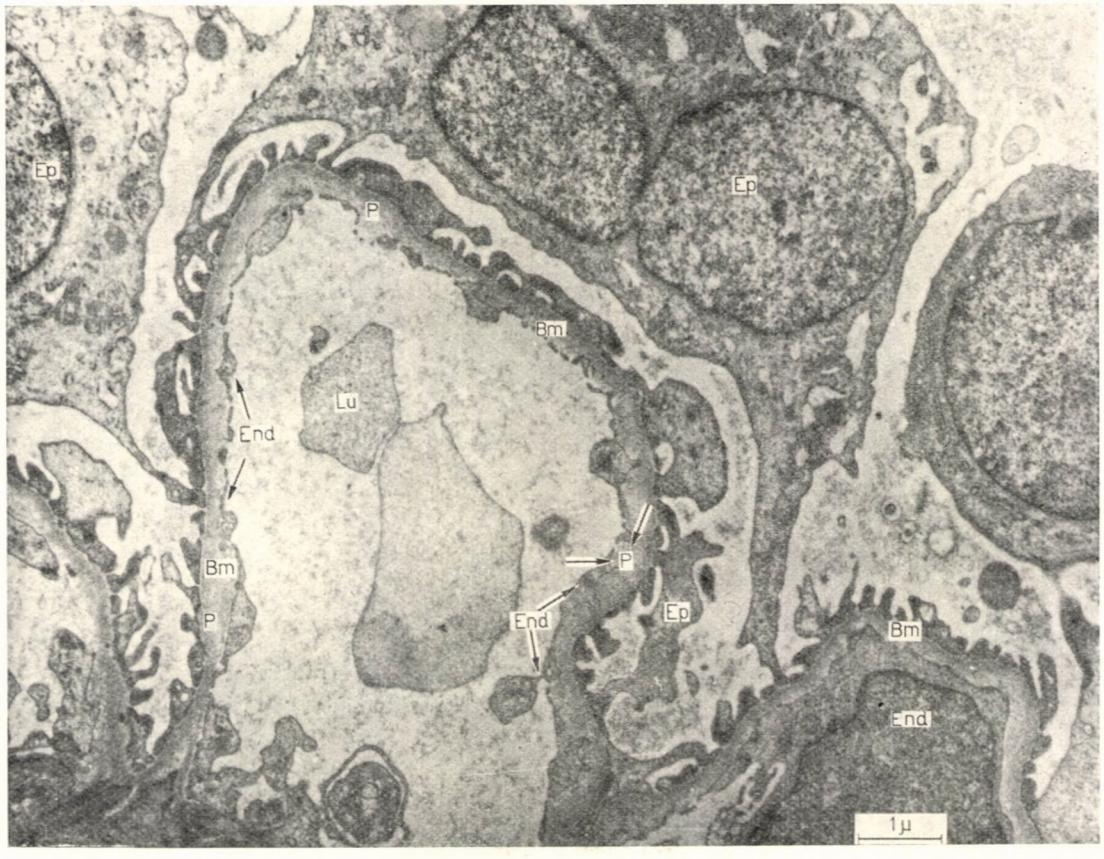

Fig. 4. Electronmicroscopical findings: the thickened basement membrane forms spine-like projections to the endothelial site of the basement membrane. On the endothelial site of the basement membrane there is an electron-dense precipitate. The foot processes are fused at many sites (×9000)

contrary to the linear type deposits which are ascribed to antibody formed against the glomerular basement membrane. Under these aspects it seemed to be interesting whether in the case of granular type immune deposition we could find data in the anamnesis regarding streptococcal infection and in the case of linear deposition, some indication of nephrotoxic factors, respectively. In fact, in 43 out of 58 cases with granular type deposition a connection between the kidney disease and some previous infection presumably of streptococcal origin could be found. In 23 out of the 43 cases glomerulonephritis began after an upper respiratory tract infection and in 20 cases the history revealed recurrent tonsillitis, or tonsillectomies. It is a point of interest that in four cases nephritis was connected with pregnancy. SLE could be ruled out in these cases all throughout and forms a separate group which has been set out in Table I.

Immunoglobulin forming linear deposition on the basement membrane was shown in 54 cases. Here, however, only in three cases an upper respiratory tract infection could be detected in the anamnesis, i.e., in a significantly lower number than in the case of granular deposition. Statistical analysis by the χ^2 test revealed that there is a correlation between granular deposition and respiratory tract infection ($p < 0.001$). In 14 cases of linear deposits there were recurrent episodes of tonsillitis in the history and in three cases the process was connected with pregnancy. No other indications of a possible nephrotoxic mechanism were noticeable.

In addition, it seemed of interest to examine the morphological types of immune deposits on the basement membrane according to age groups and the connection between the pathogenesis of glomerulonephritis and age groups.

Table II sums up the types of immune deposit according to age groups. The prevalence of the linear types of immunoglobulin deposits occurs significantly more frequently between 10 to 29 years of age, a finding which indicates the role of the antibody formed against the glomerular base-

Table II

Immunohistological findings in membraneous glomerulonephritis

Age group (years)	Granular type of IgG per cent	Linear type of IgG per cent
10—19	10.7	16.2
20—29	12.5	17.8
30—39	9.8	11.6
40—49	14.3	2.6
50—	4.4	—

ment membrane for the majority of cases of glomerulonephritis occurring in this age group. In the 30—39-year age group the incidence of linear deposits was slightly higher than that of granular deposits, whereas over 40 years of age the granular type predominated ($p < 0.05$). Beyond 50 years of age the deposits were exclusively of the granular type; this would seem to suggest that with advancing age probably the pathogenesis of glomerulonephritis after streptococcal infection plays a role.

The question then arose whether in the cases of membraneous glomerulonephritis the morphological signs of chronicity were related in any way to the pathogenesis. Therefore, we examined in every case with early signs of chronicity whether the immune deposit was of the linear or of the granular type. It was granular in 19 and linear in 9, displaying a significant difference ($p < 0.05$), in other words membraneous glomerulonephritis with signs of chronicity carries a significant prevalence of immune deposition of the granular type. This would implicate that the cases of glomerulonephritis connected with upper respiratory tract infections and a substantial number of those with streptococcal infection are more liable to chronic transformation than those of nephrotoxic origin. In fully developed chronic glomerulonephritis the immune deposits were too sparse to allow a reliable differentiation between linear or granular types.

Discussion

The investigations of DIXON et al. [2] in antigen-antibody complex nephritis have been concerned with the factors determining the transformation of glomerulonephritis into a chronic form or its acute course ending with recovery. Their results indicated that the renal changes induced in rabbits were determined by the antibody-forming capacity of the animal. In the case of a poor antibody production, each injection of antigen will result in an antigen-antibody complex with a prevalence of antigen. This complex finds access through the blood stream to the kidney thus producing glomerulonephritis. In such animals the amounts of antigen administered daily were sufficient for the neutralization of the circulating antibody. In this manner, protein administration leads to a progressive subacute or chronic glomerulonephritis, regardless whether or not the administration of the antigen is continued. If, however, the rabbits are powerful antibody producers, the experimental glomerulonephritis assumes an acute form with rapid recovery despite the continued administration of the antigen, since in the antigen-antibody complex formed by these animals it is the antibody which predominates and the prevalence of antibody makes the complex far less nephritogenic than that of antigen. From these facts SARRE [15] inferred that abundance of proteins in the diet promotes the chronic transformation of glomerulonephritis. The ques-

tion then is whether in man, too, the chronic transformation of glomerulonephritis may be related in any way to a poor antibody production in streptococcal infection. It is the aim of further studies to examine this possibility.

The role of streptococcal infection in the development of human glomerulonephritis has long been known. Recent investigations have, however, still contributed to our understanding of the part played by streptococci in the pathogenesis of this process. In particular, MARKOVITZ et al. [8] have shown that there is a common antigen of the basement membrane of human glomeruli and the nephritogenic type 12 of haemolytic Streptococcus A.

On these grounds the antibody formed as a result of streptococcal infection may well give rise to nephritis by cross-reacting with the antigen of the basement membrane, an assumption consistent with the results of experiments in which glomerulonephritis has been produced in monkeys by injection of streptococci together with human glomerular basement membrane antigen [8, 9].

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CLINICAL AND HISTOPATHOLOGICAL STUDIES IN HUMAN RENAL DISEASE

II. SIGNIFICANCE OF ISOLATED PROTEINURIA

By

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1) Compulsory screening tests may greatly contribute to the detection of monosymptomatic proteinuria.

3) On the evidence of renal biopsy and clinical observations of 39 patients with monosymptomatic proteinuria, persistent proteinuria must be regarded as a sign of renal disease. Even orthostatic proteinuria is mostly indicative of such a condition.

3) Orthostatic proteinuria in the light of observations extending to several decades carries a favourable prognosis, in contrast to persistent proteinuria, particularly in the case of intermittent and inconspicuous pathologic signs in the urinary sediment. Diagnostic studies must therefore include, in addition to renal biopsy, repeated tests of the urinary sediment even if it has been found normal at first.

4) The diagnosis of an immunohistologically active glomerulonephritis involves the necessity for clinical observation in order to ascertain its progressive or regressive tendency. In many cases, biopsy must be repeated after 3 to 4 months. In the case of a progression marked by an increase in proteinuria, the appearance of haematuria or a deterioration of the histological signs, immunosuppressive therapy should be attempted.

Until recently an incidental finding of isolated proteinuria in apparently healthy individuals had no particular diagnostic significance. It was only major proteinuria which posed a diagnostic problem unless it was associated with the nephrotic syndrome, diabetes, cardiac failure, hypertensive disease, impairment of renal function, abnormal urinary sediment or urographic findings or if there was no indication of renal disease in past history. Though transitory proteinuria may occur in healthy individuals under particular conditions such as physical exercise, mental stress, fever or major lordosis, evidence accumulating as a result of the common use of renal biopsy none the less indicates that proteinuria, however slight, is often due to renal disease even in the absence of any other sign of renal abnormality.

The pertaining data in the literature make it by no means easy to assess the clinical significance of isolated proteinuria. One of the major sources of confusion is the inconsistency of terminology. Proteinuria of apparently healthy individuals has been qualified as "asymptomatic", "juvenile", "benign", "cyclic", "physiological", "minimal", "idiopathic", "intermittent", "postural" "orthostatic", "constant" or "persistent" by the various authors. These terms are inadequate, not only because they admit of aetiological or prognostic

interpretations lacking any proof but also because the diagnostic criteria on which they are based are inaccurate, all relevant factors such as the urinary sediment, renal function, etc., with the only exception of proteinuria, being disregarded.

There is general agreement that it is expedient to divide the cases of proteinuria into two main groups, i.e. persisting (permanent) and orthostatic (intermittent) proteinuria. The analysis of our cases has also been based on this classification.

Material and method

The data expected to throw light on the question to be studied were derived from patients with monosymptomatic persistent or exclusively orthostatic proteinuria, renal biopsy having been performed with success in all of them. Only patients with normal 24-hour Addis-counts of leukocytes and erythrocytes (below 2,000,000) were included in the study.

Thirty-nine out of 289 patients were found to meet these requirements. The orthostatic group (Table I) comprised 14, the persisting group (Table II) 25 patients. As regards the history, attention was paid to the preliminary circumstances under which proteinuria had been detected. Consideration was given to the possible factors pointing to some earlier process of the urinary tract. It also seemed of importance to include all those cases in which occasional urinary samples revealed 1 to 4 erythrocytes per high power field though the 24-hour Addis-count was within the normal range. Urinary samples of this kind were generally obtained after walking or physical exercise. A transitory slight elevation of blood pressure as well as observations of oedema in the late phase of the study have also been included in the tables.

Histopathological evaluation of the biopsy material was based on light-microscopic and immunofluorescent studies (BEREGI and VARGA 1971).

Results

In the orthostatic group (Table I) the proportion of males was predominant (73.3%), mean age was 17.5 years, the upper age limit, 25 years. In more than 50% of the cases, detection of proteinuria was incidental, i.e. connected with screening or with routine investigations in connection with illness of some kind. In 6 cases there were indications of earlier disease of the urinary tract in the history. Slight microscopical haematuria after forced physical exercise was confined to two patients and three patients were found slightly hypertensive for a short period. Renal function was normal all throughout. However, it was in scarcely more than one third of the patients that the histological study failed to reveal any abnormality. In fact, membraneous glomerulonephritis was found in 6 cases. Slight microscopic abnormalities demonstrable in two cases did not fit in with the conventional histological classification (Nos 5 and 12) and in one case vascular abnormalities associated with hypertensive disease were present.

In the group of persistent proteinuria (Table II), mean age was 29.3 years, the upper age limit, 59 years. Female patients accounted for 60% of this group where proteinuria had been detected in most cases in the course

of investigations in connection with definite signs suggestive of some disease of the urinary tract. The group included patients with massive proteinuria (3.43 g/24 hours) and also the appearance of oedema was noted in the course of the observation period of 18 months following biopsy. In 28% of the cases, repeated studies of the urinary sediment revealed occasional microhaematuria. An impairment of the glomerular filtration rate was found in one case (No. 5).

In none of the cases of this group was the histological finding compatible with that of an unaffected kidney. In four cases (Nos 16, 20, 29, 38) angiopathic lesions of the hypertensive type were demonstrable in the absence of other renal abnormalities, the blood pressure being either normal or slightly elevated. In other respects the histological findings were similar to those noted in the orthostatic group; glomerulonephritis with signs of immunological activity was found in 72% of the patients.

Discussion

In the present biopsy material the figure for monosymptomatic proteinuria seems strikingly low (13.7%), in view of the fact that HANKISS (1971) found orthostatic proteinuria in 9.5% of 2846 schoolchildren. It must be emphasized that our biopsy material does not reflect the population with renal disease, not only because it includes no children, but also because biopsy had been primarily prompted by the therapeutic possibilities of the case, in other words it had been applied only for diagnostic purposes. This excluded the cases of advanced chronic glomerulonephritis, nephrosclerosis, Kimmelstiel—Wilson syndrome, or multiple myeloma from the study.

On review of the entire series it was striking to find that in 46% of the patients the detection of proteinuria had been due to screening or routine tests of some kind (schoolchildren, athletes, drivers), or to clinical examinations for some disease. This emphasizes the significance of compulsory screening tests, and also permits to measure the contributions of the health services, as illustrated by the fact that in none of the cases in the whole series has the diagnosis of proteinuria come from a tuberculosis or diabetes clinic. The other point of significance is the fact that the development of proteinuria is often insidious. On the other hand, orthostatic proteinuria may persist even after the cure of acute nephritis as illustrated by the history of two patients in the series (Nos 3, 7). In the cases of persistent proteinuria there were indications of a diffuse renal disease in the history and in this group the fortuitous discovery of proteinuria was rarer than in the orthostatic group.

A striking fact emerging from the histological study was the presence of organic renal disease in all of the cases of persistent, and in two thirds of those of intermittent proteinuria. It was in three cases only (Nos 5, 13, 28)

Table I

Orthostatic proteinuria

Patient, No., sex	Age	Nature and time of study leading to detection of proteinuria	History, symptoms	Sign of renal disease			Hypertension	Histological evidence		Note
				Orthostatic proteinuria	Intermittent erythrocyturia	Oedema		Histology	Immunohistology	
1 F	15	Clinical examination one month earlier	upper respiratory catarrh, low-back pain one month earlier	heavy precipitate	2 to 3 erythrocytes	—	—	negative	not performed	—
2 M	18	Screening (school) one year earlier	recurrent tonsillitis	slight opalescence	—	—	—	negative	not performed	—
3 F	16	Clinical examination four months earlier	facial oedema, hypertension, proteinuria four months earlier	slight opalescence	1 to 2 erythrocytes	—	—	negative	negative	—
4 M	17	Clinical examination one year earlier	hypertensive symptoms one year earlier	opalescence	—	—	170/100	nephroangiopathy	negative	—
5 M	19	Clinical examination five years earlier	low-back pain, hypertension five years earlier	slight opalescence	—	—	—	focal glomerular hyalinosis	not performed	at time of biopsy, 7 yrs. later persistent proteinuria, in sediment 5 to 6 erythrocytes, blood pressure 170/100 mm Hg
6 F	25	Screening (school) 11 years earlier	—	heavy precipitate	—	—	—	membraneous glomerulonephritis	linear IgG	—

7 M	16	Clinical examination three years earlier	facial oedema, hypertension, proteinuria three years earlier	opalescence	—	—	—	membraneous glomerulonephritis	linear IgG and C'	—
8 M	20	Accidental finding six years earlier	—	opalescence	—	—	—	membraneous glomerulonephritis	linear IgG and C'	—
9 M	16	Accidental finding six years earlier	—	opalescence	—	—	150/90	membraneous glomerulonephritis	granular IgG and C'	—
10 M	17	Accidental finding five months earlier	—	heavy precipitate	—	—	150/90	membraneous glomerulonephritis	sparse IgG and C'	—
11 M	21	Routine blood donor tests five months earlier	—	slight opalescence	—	—	—	membraneous glomerulonephritis	granular IgG and C'	—
12 M	15	Accidental finding four years earlier	microhaematuria, persistent proteinuria four years earlier	heavy precipitate	—	—	—	negative	negative	—
13 M	17	Accidental finding six years earlier	—	opalescence	—	—	—	focal interstitial fibrosis	negative	—
14 M	15	Clinical examination two months earlier	recurrent tonsillitis	opalescence	—	—	—	negative	negative	urography in the erect posture: marked nephroptosis

1. Orthostatic proteinuria denotes positivity of sulphosalicylic test obtained after walking.
2. Intermittent erythrocyturia refers to the maximum number of erythrocytes per high power field upon physical exercise with a normal Addis-count, the urinary sediment having been negative at least on two previous occasions.
3. In the column "Hypertension" only the elevated blood pressure values have been given.
4. Immunohistology denotes the character (linear or granular) of Ig and complement deposits.

Table II

Persistent proteinuria

Patient, No., sex	Age	Nature and time of study leading to detection of proteinuria	History, symptoms	Sign of renal disease			Hypertension	Histological evidence		Note
				Orthostatic proteinuria	Intermittent erythrocyturia	Oedema		Histology	Immunohistology	
15 F	44	Accidental finding two years earlier	haematuria, proteinuria two years earlier	slight opalescence	—	—	140/90	diffuse proliferative glomerulonephritis	not performed	—
16 F	51	Clinical examination two years earlier	crural oedema, fatigue two years earlier	heavy precipitate	—	+	—	nephroangiopathy	not performed	—
17 M	28	Routine medical test for driving licence	—	heavy precipitate	—	—	—	diffuse proliferative glomerulonephritis	not performed	—
18 F	35	Clinical examination one year earlier	signs of acute nephritis one year earlier	opalescence	1 to 3 erythrocytes	—	—	membraneous glomerulonephritis	scanty IgG	—
19 M	45	Clinical examination five months earlier	dysuria five months earlier	slight opalescence	2 to 4 erythrocytes	—	150/90	membraneous glomerulonephritis	granular IgG	—
20 M	59	Clinical examination four months earlier	genaralized oedema four months earlier	heavy precipitate	—	+	150/90	nephroangiopathy	negative	C_{cr}: 27.5 ml/min Se. cr. 2.55 mg%
21 F	30	Accidental finding six months earlier	—	opalescence	—	—	—	focal glomerulonephritis	fibrin	—

22 F	46	Clinical examination 16 years earlier	toxaemia of pregnancy 16 years earlier	0.192 g	—	—	—	membraneous glomerulonephritis	patchy deposits of IgG and C'	—
23 M	25	Clinical examination six months earlier	low-back pain six months earlier	0.500 g	1 to 3 erythrocytes	—	—	membraneous glomerulonephritis	linear IgG and C'	—
24 F	28	Clinical examination six months earlier	oedema six months earlier	0.840 g	—	—	—	membraneous glomerulonephritis	granular IgG and C'	—
25 F	15	Clinical examination two weeks earlier	low-back pain, headaches, subsequent to tonsillitis two weeks earlier	0.264 g	—	—	150/100	membraneous glomerulonephritis	linear IgG and C'	—
26 F	34	Accidental finding one month earlier	episodes of tonsillitis, cystitis two years earlier	0.155 g	—	—	145/90	membraneous glomerulonephritis	linear IgG and C'	—
27 M	15	Clinical examination four months earlier	tonsillitis four months earlier	0.410 g	—	—	—	membraneous glomerulonephritis	linear IgG, C', deposits of fibrin	—
28 F	25	Routine sports medical examination nine years earlier	recurrent tonsillitis	opalescence	—	—	—	focal glomerular hyalinosis, interstitial fibrosis	negative	—
29 M	35	Routine medical tests for driving-licence one year earlier	—	1.020 g	2 to 3 erythrocytes	—	145/95	nephroangiopathy	negative	—

Table II (cont.)

Patient, No., sex	Age	Nature and time of study leading to detection of proteinuria	History, symptoms	Sign of renal disease			Hypertension	Histological evidence		Note
				Orthostatic proteinuria	Intermittent erythrocyturia	Oedema		Histology	Immunohistology	
30 F	35	Pregnancy test five years earlier	toxaemia of pregnancy five years earlier	1.160 g	1 to 2 erythrocytes	—	—	membraneous glomerulonephritis	granular IgG and C'	—
31 M	16	Screening (school) three months earlier	—	1.169 g	—	—	—	membraneous glomerulonephritis	linear IgG and C'	—
32 F	26	Clinical examination two months earlier	low-back pains two months earlier	1.129 g	—	—	—	membraneous glomerulonephritis	linear IgG and C'	—
33 F	18	Clinical examination two years earlier	low-back pains, dysuria two years earlier	0.520 g	1 to 4 erythrocytes	—	—	membraneous glomerulonephritis	linear IgG and C'	—
34 F	16	Routine sports medical examination two years earlier	—	0.203 g	1 to 2 erythrocytes	—	—	membraneous glomerulonephritis	linear IgG and C'	—
35 M	14	Clinical examination one year earlier	signs of acute nephritis one year earlier	0.928 g	—	—	—	membraneous glomerulonephritis	linear IgG and C'	—

36 F	21	Clinical examination two months earlier	signs of acute nephritis two months earlier	2.500 g	—	—	—	membraneous glomerulonephritis	linear IgG and C'	—
37 M	27	Accidental finding five years earlier	—	3.420 g	—	—	—	membraneous glomerulonephritis	patchy deposits of IgG and C'	—
38 F	21	Clinical examination two years earlier	crural oedema two years earlier	2.640 g	—	+	—	nephroangiopathy	negative	—
39 M	18	Clinical examination eight years earlier	gross haematuria eight years earlier	0.154 g	—	—	—	membraneous glomerulonephritis	linear IgG and C'	—

1. 24-hour urinary excretion of protein on the basis of biuret-reaction.
2. 3. and 4. see Table I.

that the changes found did not fit in with any of the well-established types of renal disease. The interstitial fibrosis and focal-segmental hyaline degeneration of glomeruli demonstrated in these cases may have represented residual lesions after some previous infectious or toxic damage, or of cured glomerulonephritis. The histological findings in all other patients were consistent with some well-defined condition such as membraneous or proliferative glomerulonephritis, focal nephritis, interstitial nephritis or vascular nephropathy. The data in the literature on the results of biopsy in this kind of proteinuria are basically consistent with the present observations; in addition, they mention the possibility of myeloma and of amyloidosis (POLLAK et al. 1958, MUTH 1965, ANTOINE et al. 1966, ROBINSON et al. 1961, PHILLIPPI et al. 1966, MOREL-MAROGER et al. 1967, THOMPSON et al. 1970).

As to the relationship between the type of proteinuria and that of renal lesion, it is found that both types of proteinuria under study may be associated with the same type of lesion. The angiopathy group deserves none the less particular attention, it being characterized by a lamellar elastosis of the preglomerular vessels. Lesions of this type were confined to a single case of orthostatic proteinuria (No. 4), where this finding was amply accounted for by a long hypertensive episode experienced by the patient at least one year before biopsy, and reflected by typical changes in the eyegrounds. All other cases with histological evidence of nephroangiopathy revealed massive proteinuria with a normal blood pressure (Nos 16, 20, 29 38). MOREL-MAROGER et al. (1967) found intimal proliferation and hyalin and fibrin depositions in the arteries with no light-microscopical evidence of glomerular damage in one third of their cases of monosymptomatic proteinuria, none of the patients being diabetic or hypertensive, similarly to those of the present series. All that seems to support the observations of SZINAY et al. (1963) and that of GÖMÖRI et al. (1968) that the severity of vascular changes of the affected kidney is often unrelated to the duration of hypertension. Recently, TAKEBAYASHI et al. (1971) have suggested the possibility of an immune pathomechanism for the interpretation of the vascular lesions of the kidney. It adds to the problems that the relationship between the arterial lesions and the pathomechanism of proteinuria is not clear. Electronmicroscopic examinations might perhaps throw light on the nature of glomerular lesion.

Before discussing the clinical significanc of monosymptomatic proteinuria, it must be stressed that the histological changes found in our group of orthostatic proteinuria show a far higher figure than those noted by ROBINSON et al. (1961). The difference may be due to the fact that in the first period of the study we abstained from biopsy in those cases where urography performed in the erect posture revealed a distinct nephroptosis. As a result, a number of the cases of proteinuria resulting from actual venous congestion may have been excluded from the series. On the other hand, the percentage

of organic renal disease found in our group of persistent proteinuria corresponds to the figures of other authors (POLLAK et al. 1958, PHILLIPPI et al. 1966, MOREL-MAROGER 1967), with the exception of MCLAINE and DRUMMOND (1970) who found no renal disease in their series of proteinuric children.

Predictions of the clinical course and survival on the ground of the histological appearance are fraught with difficulties, since it is hardly ever possible to tell whether a given lesion is progressive, static or regressive. This gives particular significance to long-term observations.

We owe the first well-documented report on a follow-up of patients with orthostatic proteinuria over a period of five years to LECOCQ et al. (1966). At the end of this period proteinuria was still present in 70% of the cases without any sign of functional impairment of the kidney. However, slight changes in the urinary sediment of a few patients (microhaematuria, casts) made the authors cautious in their prediction as to survival. The same patient material at the end of a ten-year period provided more satisfactory evidence (THOMPSON et al. 1970); though proteinuria of the orthostatic or persisting type was still present in 40% of the cases, none of these patients displayed any clinical or laboratory evidence of progressive renal disease. ANTOINE et al. (1966, 1969) reported on a still longer follow-up of patients with persisting proteinuria. Despite definitely abnormal histological findings, signs of functional impairment were confined to two cases known to be proteinuric for 15 and 18 years, respectively. Basically the same results emerge from the observations of LEVITT (1967) pursued over still longer periods, though without biopsy, in 185 college students with proteinuria. Their reexamination 37 to 45 years later allowed the conclusion that, in the absence of other signs of renal disease, intermittent proteinuria is a harmless condition not leading to renal failure. If, however, proteinuria is permanent or associated with a pathologic urinary sediment, the outcome is unfavourable, death from renal failure having occurred in 57% of the cases.

Though the data derived from the present material give little insight into the possible changes of the process in time, we may none the less allege in support of the harmless nature of monosymptomatic proteinuria that in the orthostatic group we had an instance of proteinuria dating 11, and in the persistent group one dating 16 years back without any clinical sign of functional impairment of the kidney. On the other hand, in case No. 5 the histological changes were slight and indistinct, yet the diagnosis of orthostatic proteinuria which had been justified seven years earlier, was no longer tenable in view of the constant proteinuria found in association with a pathologic urinary sediment and with arterial hypertension at the time of biopsy. No improvement was noted in the cases of nephroangiopathy during the two years period of observation. On the contrary, oedema appeared in three cases, arterial hypertension of minor degree was found in case No. 29 at the end of one year,

and the renal function had been affected at the very outset in case No. 20. The final outcome of proteinuria accompanying nephroangiopathy is obviously less favourable than that of other isolated protein-losing conditions.

As in any diffuse renal disease, in renal disease associated with isolated proteinuria the possibility of immunosuppressive therapy must be considered. This obviously does not apply to pyelonephritis or focal nephritis or to renal conditions of indistinct morphological pattern which may represent irreversible sequelae of some earlier infection, in particular of glomerulonephritis.

From the observations published it can be concluded that, while in the prospect of ten to fifteen years, carefully selected cases of monosymptomatic proteinuria carry a favourable long-term prognosis, a pathologic urinary sediment, however inconspicuous, may have a grave prognostic significance. In the present material the majority of patients with intermittent microhaematuria, though still inside the limits of a normal Addis-count, had an immunohistologically active, membraneous glomerulonephritis. In glomerulonephritis associated with similar morphological changes, immunosuppressive therapy is considered imperative. Obviously, it needs more than a single examination to know whether the histological changes represent a regressive post-nephritic condition, or a progressive glomerulonephritis still in the early latent stage. In some cases clinical observation for a period not longer than two weeks may be sufficient to motivate a therapy of this kind (increase in proteinuria, stabilisation of microhaematuria). Other cases may require an observation period as long as 3 or 4 months, as a persistent, even though diminishing, proteinuria and the decision for immunosuppressive therapy rests on the evidence of repeated biopsy and immunohistological studies.

The poor understanding of the pathogenesis of vascular nephropathy makes immunosuppressive therapy unsuited for this type of renal disease. Closer studies into the nature of the process are expected to open up new therapeutic possibilities.

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ACTA MEDICA

ТОМ 28 — БЫП. 3—4

РЕЗЮМЕ

ИЗУЧЕНИЕ ТРОМБОЭЛАСТОГРАФИЧЕСКОГО ПОКАЗАТЕЛЯ В СТАДИЯХ ОБОСТРЕНИЯ И РЕМИССИИ ИСТИННОЙ ПОЛИЦИТЕМИИ

Д. НАДЬ, М. МИС, Б. ШИРО и Я. СЕГЕДИ

У 55 больных истинной полицитемией проведен анализ тромбоэластографического показателя и его компонентов, а такие активности фибринолиза. В стадии обострения почти все исследовавшиеся параметры показали патологическое изменение. В фазе ремиссии эти отклонения уменьшались или отчасти нормализовались. Результаты исследований находятся в полном согласии с наблюдением, согласно которому в стадии обострения заболевания часто наблюдаются васкулярные осложнения, в то время как в фазе ремиссии они только очень редко встречаются.

ЛЕЧЕНИЕ ИСТИННОЙ ПОЛИЦИТЕМИИ ПРИ ПОМОЩИ НОВЫХ ВЕНГЕРСКИХ ЦИТОСТАТИЧЕСКИХ СРЕДСТВ

Д. НАДЬ, Ч. БАЛАЖ и Д. ПЕТРАНЬИ

В течение более чем трехлетного периода наблюдения авторы применяли для лечения 26 больных истинной полицитемией Миелобромол, а у 34 больных — Цитостоп, при кратковременной даче больших доз. В первой группе у 23 из 26 больных, а во второй группе у всех 34 больных удалось достигнуть полной ремиссии. В двух случаях наблюдали обратимое осложнение, которое можно было привести в связь с терапией. Результаты сопоставлены с результатами, полученными в связи с применением ^{32}P, и подытоживаются руководящие принципы лечения истинной полицитемии.

ИЗУЧЕНИЕ ВЕНТРИКУЛЯРНОГО ПРОВЕДЕНИЯ РАЗДРАЖЕНИЯ МЕТОДОМ ВЕКТОРКАРДИОГРАФИИ У БОЛЬНЫХ С ИМПЛАНТИРОВАННЫМ ЭЛЕКТРОСТИМУЛЯТОРОМ СЕРДЦА

П. КЕНЕДИ

Векторокардиографическое исследование больных с имплантированным электростимулятором является

1. пригодным методом для сравнения векторокардиографических систем;
2. при его помощи можно подробно изучать искусственное раздражение;
3. точно определить локализацию раздражающего электрода;
4. открывается возможность для электрофизиологического анализа проведения раздражения в опыте на человеке.

ОПРЕДЕЛЕНИЕ ОТДЕЛЬНЫХ НЕКОНЪЮГИРОВАННЫХ, СУЛЬФАТНЫХ И ГЛЮКУРОНОЗИДНЫХ ЭФИРОВ 17-КЕТОСТЕРОИДОВ В ЧЕЛОВЕЧЕСКОЙ МОЧЕ

Т. ФЕХЕР

Сообщается метод для определения отдельных метаболитов 17-кетостероидов в неконъюгированной форме или в форме эфира сульфата или глюкуронозида в человеческой моче. После частичного гидролиза и экстракции разделение отдельных метаболитов проводилось методом хроматографии на бумаге, и количественное определение при помощи микрореакции Циммермана.

Согласно результатам исследования здоровых лиц в неконъюгированной фракции мочи господствуют главным образом этиохоланолон, 11—ОН- и 11—кето-этиохоланолон, в фракции сульфата — дедигроэпиандростерон и в большем соотношении 5 α-стероиды, а в фракции глюкуронозида — 5 β-производные 21-окси- и 11-дезоксиметаболитов.

Сообщаются также изменения выделения неконъюгированных эфиров и эфиров сульфата и глюкуронозида после введения АКТГ, метопирона, тестостерона и дегидроэпиандростерона.

ТОНОГРАФИЧЕСКОЕ ИССЛЕДОВАНИЕ ГЛАЗ С КОМБИНИРОВАННОЙ ДИСТРОФИЕЙ РОГОВИЦЫ ФУКСА

Д. ИМРЕ

На 17 глазах с комбинированной дистрофией роговицы Фукса при определении тонографическим методом средняя величина минутного объема камерной влаги составляла $0{,}49 \pm 0{,}37$ мм³/мин. Вопреки источникам ошибок метода эту величину можно рассматривать как признак выраженного уменьшения. Пониженное образование камерной влаги может привести к нарушению питания роговицы, и очень вероятно, что совместное наличие изменения заднего барьера (cornea guttata) и понижения образования камерной влаги обуславливают развитие комбинированной дистрофии роговицы Фукса.

РАЗМЕР ПРОТЕИНОВОГО ПРОСТРАНСТВА В НЕВРЕДИМОЙ ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЕ И ПРИ ОСТРОМ ПАНКРЕАТИТЕ У КРЫС

Действие лигирования грудного протока на смертность от панкреатита

М. ПАПП, Л. ЧАКИ и Ш. ОРМАИ

На белых крысах породы Выстар или штамма CFE было изучено протеиновое пространство в поджелудочной железе при помощи меченого Cr^{51} протеина или $У^{131}$-альбумина, и определено содержание сухого вещества в поджелудочной железе через три часа после введения в грудной проток 5 мг трипсина или растворителя. Инъекция была комбинирована также лигированием грудного протока. Смертность животных изучалась через сутки после указанных вмешательств. Было установлено, что меченое протеиновое пространство невредимой поджелудочной железы составляет 23 мл/100 г влажной поджелудочной железы. Дача трипсина повышает эту величину до двукратного исходного значения. Без вмешательства в 100 г влажной поджелудочной железы сухое вещество составляет 27%, после введения трипсина в 100 г влажного панкреаса содержание сухого вещества понижается до 16%. Через 3 часа после лигирования грудного протока не наблюдается изменения размера отека поджелудочной железы, а после введения растворителя меченое протеиновое пространство панкреаса увеличивается на 150%. По истечении 24 часов под влиянием лигирования грудного протока смертность животных, получивших только трипсин, повышается на трох-четырехкратное исходной величины.

ВЫЗВАНИЕ АВТОЛОГИЧНОГО ИММУННОГО КОМПЛЕКСНОГО ГЛОМЕРУЛОНЕФРИТА ПРИ ПОМОЩИ ИНЪЕКЦИЙ ГЕТЕРОЛОГИЧЕСКОГО ПОЧЕЧНО-ТУБУЛЯРНОГО АНТИГЕНА

А. Н. НАГИ М. Б., Б. С., А. З. БАРАБАШ, Ф. АЛЕКСАНДЕР и Р. ЛАННИГАН

Автологичный иммунный комплексный (АИК) гломерулонефрит был вызван у крыс при помощи повторных внутрибрюшинных инъекций человеческого почечно-тубулярного антигена. Развитие поражения почек привело к протеинурии и заболеванию печени, подобно тому, как и при экспериментально вызванном автоиммунном нефрозе (типа Геймана) у крысы и при человеческой перепончатой нефропатии. Выдвигается гипотез, быть может, объясняющий развитие этого прогрессирующего заболевания почек в указанных экспериментальных условиях.

ДЕЙСТВИЕ АДЕНОЗИНТРИФОСФАТА — АТФ — НА ЦЕРЕБРАЛЬНОЕ КРОВООБРАЩЕНИЕ И НА ОБМЕН ВЕЩЕСТВ МОЗГА ПРИ ИШЕМИЧЕСКИХ ПАТОЛОГИЧЕСКИХ ПРОЦЕССАХ

Ф. ШОЛЬТИ, Д. ХАДИЕВ, Э. ВИТЧЕВ и Й. НАДЬ

Авторами было изучено действие аденозинтрифосфата (20 мг, внутривенно) в 30 случаях церебральной ишемии цереброваскулярного происхождения, оказанное на кровообращение и на обмен веществ головного мозга. В экспериментах по определению церебрального кровообращения авторы использовали метод двойной пункции внутренней яремной вены и метод венозной дилюции изотопа при помощи противотока. После дачи АТФ в части случаев существенно возросло церебральное сосудистое сопротивление, в общем однако ни церебральное кровообращение, ни сосудистое сопротивнение мозга не показали достоверных изменений. После совместного введения АТФ + норадреналина — в форме внутривенного капельного вливания — однако, церебральное кровообращение достоверно повысилось, а сосудистое сопротивление мозга достоверно понизилось. Поглощение и использование глюкозы и O_2 в мозге не показали изменения, ни под влиянием АТФ, ни после одновременного введения АТФ + норадреналина.

ИЗУЧЕНИЕ НЕЭСТЕРИФИЦИРОВАННЫХ ЖИРНЫХ КИСЛОТ ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ ГИПЕРТОНИИ У КРЫС

М. Л. МИХАЙЛОВ

Рисунок газового хроматогрифического анализа свободных, неэстерифицированных жирных кислот (СЖК) в плазме крови был изучен на двух моделях экспериментальной гипертонии крыс — нефрогенной и нейрогенно-интероцептивной гипертонии — и результаты были сопоставлены с данными животных с нормотонией. При гипертонии рисунок СЖК показывает характерные изменения.

1. Содержание насыщенных СЖК повышается, главным образом содержание пальмитина и стеариновой кислоты;
2. Содержание ненасыщенных СЖК уменьшается диаметрально противоположно содержанию насыщенных СЖК, особенно концентрация олеиновой кислоты, пальмитиновой кислоты и линолевой кислоты.

На модели нефрогенной гипертонии изменения, указывающие на повышенное участие механизма ренин-ангиотензина были более выраженными.

ИЗМЕНЕНИЕ КИНЕТИКИ ЙОДА В ОРГАНИЗМЕ ПОД ВЛИЯНИЕМ ВВЕДЕНИЯ РЕОПИРИНА

К. БАРТА

Автором было изучено действие реопирина на функцию щитовидной железы путем определения коэффициента поглощения и мобилизации йода, характерных для йодной кинетики организма. Результаты исследований следующие:

Стойкий уровень медикамента в зависимости от применяемой дозы, всегда достоверно тормозиет функцию щитовидной железы.

Предварительное применение фенибутазона не влияет на йодный обмен щитовидной железы.

Ингибиторное действие нельзя приписать повышению введения TSH, так как не наблюдалось повышения веса щитовидной железы.

Когда действующее вещество попадает в организм после окончания поглощения радиойода, то наблюдается мнимая гипертиреотическая мобилизация. Однако по свидетельству данных определения активности плазмы это характерно только для выступающего из щитовидной железы йода или для соотношения активного-неактивного йода в сыворотке.

ИЗУЧЕНИЕ ФУНКЦИИ КРОВООБРАЩЕНИЯ У БОЛЬНЫХ ИСТИННОЙ ПОЛИЦИТЕМИЕЙ

II. Действие симпатико и парасимпатико-миметических веществ на периферическое кровообращение

П. ДАРОЦИ, Д. НАДЬ, и Я. СЕГЕДИ

Авторами было изучено действие симпатико- и парасимпатико-миметических веществ на периферическое кровообращение у 40 больных истинной полицитемией. Было установлено, что у большинства больных полицитемией можно выявить склонность к сосудистым спазмам при повышенной чувствительности к норадреналину. У больных полицитемией эти явления выявляемы как в стадии обострения, так и в стадии ремиссии, и они едва поддаются лечению. Следовательно прекращение жалоб больных зависит от успешного лечения основной болезни.

ИЗМЕНЕНИЯ ЛИПОИДНЫХ ФРАКЦИЙ СЫВОРОТКИ И ПЕЧЕНИ У КРЫСЫ ПОСЛЕ МЕСТНОГО ОБЛУЧЕНИЯ ПЕЧЕНИ ДОЗОЙ 4000 р

Л. ШОЛТЕС и Г. ГОТТВАЛЬД

После местного облучения печени крысы 4000 р были изучены изменения липоидных фракций сыворотки и печени. В течение первых семи дней после облучения ни в одной из липоидных фракций сыворотки не наблюдалось достоверного изменения. В то же время в печени в существенной мере понизилось содержание холестерина. Это изменение оказалось обратимым и оно нормализовалось в течение 42 дней. Фракции фосфорлипоидов и эстерифицированной жирной кислоты печени не показали изменения. Начиная с седьмого дня после облучения концентрация фосфорлипоидов, по истечении десять дней концентрация эстерифицированной жирной кислоты и после 28-го дня концентрация холестерина достоверного повысились. Понижение холестерина в печени, наблюдаемое через 72 часа после облучения позволяет сделать вывод о непосредственном поражении этого органа. На повышение концентрации сывороточных липоидов, наблюдаемое позже, могут влиять и другие факторы.

ЭКСПЕРИМЕНТАЛЬНОЕ ИЗУЧЕНИЕ НЕЙРОТОКСИЧЕСКОГО ДЕЙСТВИЯ МЕТОКСИ-ЭТИЛ-МЕРКУРИ-ХЛОРИДА

К. ЛЕХОЦКИ, Ш. БОРДАШ, Я. ШЕБЁК и И. БАТШКОР

Авторами было изучено на белых крысах нейротоксическое действие метокси-этилмеркури-хлорида (МЭМХ). У 18 ненаркотизированных, иммобилизированных в колодках кроликов авторы определяли скорость проведения импульса в седалищном нерве. Под влиянием дозы 6 мг МЭМХ на кг веса тела в день, введенной на протяжении десяти недель, скорость проведения импульса в седалищном нерве уменьшалась до 57% или 40% контрольных величин. Понижение скорости проведения импульса сопровождается хорошо выраженной демилинизацией нерва. Изложенные результаты предоставляют дальнейшие экспериментальные данные о нейротоксических действиях органических соединений ртути.

ОБЪЕМ ВНЕКЛЕТОЧНОГО И ВНУТРИСОСУДИСТОГО ПРОСТРАНСТВ ЖИДКОСТИ У СОБАК С РЕНО-ВАСКУЛЯРНОЙ ГИПЕРТОНИЕЙ

А. ФЕКЕТЕ, А. ФАЗЕКАШ и А. РЕНЬИ—ВАМОШ

У собак, после одностороннего лигирования почечной артерии — при невредимой почке противоположной стороны — была вызвана рено-васкулярная гипертония, которая на основе ряда особенностей подобна человеческому заболеванию. Было установлено, что в хроническом периоде гипертонии достоверно уменьшены как пространство распределения тиосульфата, так и объем плазмы и крови, по сравнению с контрольными собаками с нормотонией. Наблюдаемое уменьшение объема внеклеточного и внутрисосудистого пространств авторы рассматривают как последствие характерной для гипертонического состояния перестройки восприятия обьема.

ИЗУЧЕНИЕ ПРИРОДЫ АНТИТЕЛ ПРОТИВ LONG-ACTING THYROID STIMULATOR (LATS)

Я. ФЕЛЬДЕШ, Й. МАКО, Ч. БАН и Е. ГЕСТЕШИ

При применении методики, служащей для отделения комплекса антиген-антитело, ействие LATS—IgG остается неизменным. Это наблюдение говорит против природы комплекса антиген-антитело LATS—IgG.

Противотела, образовавшиеся против микросомальной фракции эпителиальных клеток щитовидной железы встречаются в случаях LATS-положительности чаще, чем в LATS-отрицательных случаях. В то же время между содержанием LATS в плазме и титром микросомальных противотел не наблюдается связи. При применении хроматографии на столбе, пригодной дли обособления так наз. «медленных» и «быстрых» IgG, LATS и CF присутствуют только в «медленном» типе IgG, в то время как противотела, образовавшиеся против тиреоглобулина, присутствуют в обоих типах IgG. Этот результат указывает на то, что различные противотела относятся к различным подклассам IgG. Тот факт, что LATS и микросомальные противотела относятся к одному и тому же подклассу IgG, обьясняет, почему обособление этих противотел является такой трудной задачей.

КЛИНИКА ЛАКТАЦИДОЗА

Э. ХОЛЛЕНДЕР

Лактат-ацидоз является одной формой метаболических ацидозов, для которой наряду с увеличением содержания молочной кислоты в крови характерно повышение концентрации пировиноградной кислоты и коэффициента молочная кислота/пировиноградная кислота, и часто также понижение содержания бикарбоната и pH артериальной крови.

Изменения величин молочной кислоты, пировиноградной кислоды и кислотно-щелочного равновесия крови были изучены в 230 случаях. Лактацидоз присоединился к известному, по большинстве случаев тяжелому основному заболеванию. Клинические симптомы наблюдались при уровне молочной кислоты выше 5 мэкв/л. На переднем плане клинической картины находились нарушения дыхания и сознания. Как симптом, указывающий на лактацидоз, автор рассматривает сравнительно более низкую концентрацию хлора в плазме, чем можно полагать на основе величин содержания натрия и калия. Тяжелый молочнокислый ацидоз — состояние, трудно поддающееся терапевтическому влиянию, и имеет плохой прогноз.

СТИМУЛИРОВАНИЕ ЛИМФАТИЧЕСКИХ ОРГАНОВ IN VIVO

Д. САС

У кроликов пролонгированным внутривенным введением гетерогенной крови (ГК) и фитогемагглютинина (ФГА) удалось вызвать характерное изменение всех (периферических и центральных) лимфатических органов. Сущность изменения следующее: изменение распределения лимфоцитов по размерам, перевес крупных лимфоцитов, образование бластов и появление митозов. Это изменение соответствует изменению, возникающему в культурах лимфоцитов под влиянием специфических и неспецифических митогенов. Изменение in vivo можно поставить в параллель с картинами изменений лимфатических узлов, известных из человеческой патологии, в которых кроме специфических изменений также видно преобладание крупных лимфоцитов и образование бластов. Из всего сказанного можно сделать вывод, что изменение, возникающее в результате стимулирования лимфоцитов, собственно говоря, является реакцией лимфоцитов.

Наряду с указанным выше изменением, наблюдаемым во всех лимфатических органах, появляются и такие изменения, которые, предположительно, связаны с особенностями отдельных органов. Такими изменениями являются гемопоэз в селезенке и в миндалинах, тучноклеточная реакция в зобной железе. Эти изменения, быть может, связаны со способностью бластов к дальнейшей пролиферации.

КЛИНИЧЕСКИЕ И ГИСТОПАТОЛОГИЧЕСКИЕ ИССЛЕДОВАНИЯ ПРИ ТЕРАПЕВТИЧЕСКИХ ПОЧЕЧНЫХ БОЛЕЗНЯХ

I. Некоторые вопросы взаимосвязи патогенеза гломерулонефрита и возраста

Э. БЕРЕГИ и И. ВАРГА

Подытоживая полученные нами результаты, можно констатировать, что в ходе наших исследований на основании морфологической картины и данных анамнеза мы можем подтвердить те данные, которые указывают на то, что появляющееся на базальной перепонке клубочков иммунное отложение зернистого характера в значительной части случаев возникает в результате инфекции, предположительно стрептококковой инфекции, в то время как в случае отложения линейного характера нельзя было выявлять такой связи. Наряду с этим можно было констатировать, что в молодом возрасте (от 10 до 29 лет) достоверно чаще наблюдалось иммунное отложение, появлявшееся в линейной форме, что указывало на то, что нефрит в этом возрасте в большинстве случаев носит нефротоксический характер. В более пожилом возрасте (старше 40 лет), в противоположность этому достоверно чаще можно было выявлять иммунное отложение зернистого характера на базальной перепонке клубочков, чем отложения линейного характера, в то время как у больных старше 50 лет на базальной перепонке клубочков обнаруживались гамма-глобулин и комплемент зернистого характера. В связи с этим морфологическим наблюдением возникает вопрос, что в более пожилом возрасте чаще имеет место инфекция верхних дыхательных путей и возникающий -вероятно после стрептококковой инфекции гломерулонефрит. Морфологические наблюдения указывают, наряду с этим, на то, что при мембранозном гломерулонефрите морфологические признаки роничности процесса встречались чаще в случае отложения зернистого характера, что указывает на то, что нефрит, возникающий после

инфекций верхних дыхательных путей, предположительно стрептококкового характера, более склонен к переходу в хроническую форму, чем нефрит нефротоксической этиологии.

Возникновение человеческого гломерулонефрита не может быть сведено к одному лишь фактору, вероятно в его возникновении роль играет ряд факторов. Целью настоящей работы было указать на то, какая возможность имеется из материала биопсии на основании морфологической картины делать выводы относительно фактора, индуцировавшего процесс.

КЛИНИЧЕСКИЕ И ГИСТОПАТОЛОГИЧЕСКИЕ ИССЛЕДОВАНИЯ ПРИ ТЕРАПЕВТИЧЕСКИХ ПОЧЕЧНЫХ БОЛЕЗНЯХ

II. О значении изолированной протеинурии

И. ВАРГА, Э. БЕРЕГИ и Б. КЕНЕЗ

1. В деле распознавания изолированной протеинурии большую роль смогут играть обязательные массовые профилактические обследования.

2. По данным гистопатологического (почечная биопсия) исследования и клинического обследования 39 больных изолированной протеинурией, постоянное выделение белка может рассматриваться почечной болезнью. Ортостатическая протеинурия также лишь в меньшей части случаев является безвредным состоянием.

3. Согласно работам, основывающимся на длительных наблюдениях прогноз ортостатической протеинурии в перспективе нескольких десятилетий благоприятный. Если же протеинурия постоянная, особенно если в осадке мочи время от времени имеются незначительные патологические явления, прогноз неблагоприятный. Поэтому, наряду с проведением биопсии, важно тщательное, повторное исследование осадка мочи и в том случае, если при первом исследовании результат отрицательный.

4. В случае иммуногистологически активного гломерулонефрита на основании клинического наблюдения или на основании результата повторенной через 3—4 месяца биопсии делается вывод относительно регрессивного или прогрессивного изменения процесса. В случае усиления протеинурии, появления гематурии или ухудшения гистологической картины нужно попытаться с иммуносупрессивным лечением.

ANNOUNCEMENT OF AN IAEA SYMPOSIUM

Title:	Medical Radioisotope Scintigraphy
Date:	23—28 October, 1972
Location:	Monaco
Organizers:	International Atomic Energy Agency Kärntnerring 11—13, 1010 Vienna, Austria
Scientific Secretaries:	Dr. E. H. Belcher and Dr. T. Nagai Medical Applications Section

The programme for this Symposium, the fourth to be organized by the International Atomic Energy Agency in the subject field, will cover all aspects of the scintigraphic technique and its applications, but will give particular attention to new advances in technique, in particular the introduction of new radiopharmaceuticals such as those labelled with short-lived radioisotopes, the further refinement of instrumentation and the use of computers for data processing and analysis.

Further information, participation forms and forms for submission of a paper intended for presentation at the Symposium may be obtained from national authorities for atomic energy matters. Abstracts of such papers must be submitted through these authorities so as to reach the International Atomic Energy Agency before 26 May, 1972.

CHIRURGENKONGRESS MIT INTERNATIONALER BETEILIGUNG

12.—14. Juli 1973, Bratislava

Tschechoslowakische Gesellschaft für Chirurgie der Tschechoslowakische Medizinische Gesellschaft J. E. Purkyně

Themen:

1. Chirurgie der akuten und chronischen Pankreatisis
2. Polytraumatismus (lebensgefährliche Verletzungen)
3. Angiochirurgie
4. Varia

Sekretariat:

Slowakische Medizinische Gesellschaft
Kongressbüro
Bratislava, Mickiewiczova 18, ČSSR

SECOND INTERNATIONAL SYMPOSIUM ON CANCER DETECTION AND PREVENTION

Bologna (Italy), April 9—12th, 1973

The Second International Symposium on Cancer Detection and Prevention, promoted by the International Study Group on Detection and Prevention of Cancer (De P Ca), under the auspices of the International Union against Cancer (UICC) and of the International Agency for Research on Cancer (IARC) of the World Health Organisation, and organized by the Istituto di Oncologia "F. Addarii" and Bologna Cancer Centre, will be held in Bologna from April 9 to 12, 1973.

The objectives of the Symposium are the discussion and the evaluation of the most recent knowledge and results in the field of cancer prevention and detection.

The program includes:

1. Lectures on:

— Cancer prevention by environmental control
— Occupational carcinogenesis
— Host factors in oncogenesis
— Recent advances and perspectives of automation in cytology
— Aspects of experimental carcinogenesis related to cancer prevention
— Evaluation of cancer control measures (Report from the Sheffield UICC Meeting)
— Carcinogenic hazards from drugs
— The possible importance of nitrosamines as carcinogens in humans

2. Panels on:

A) Scientific bases and possibilities of detection and prevention of:
— Cancer of the breast
— Cancer of the lung
— Gastric cancer
— Cancer of the colon and rectum
— Cancer of the urinary tract
— Uterine cervix carcinoma

B) Public Health aspects in Cancer Control

C) Recent advances on tumours with particular racial and geographic distribution (oral cancer, Burkitt's lymphoma, nasopharyngeal cancer, esophageal cancer, liver cancer).

3. Proferred paper sessions on the same topics as those of panels and on other fields of cancer detection and prevention.

Workshops for registered participants are planned the two days after the Symposium (April 13 and 14) on: Epidemiological techniques, cell pathology, thermography, testing of environmental carcinogens, cytogenetics, needle aspiration cytology, gastric cytology.

Official languages: English and French

Secretariat:

2nd International Symposium on Cancer Detection and Prevention INSTITUTO DI ONCOLOGIA "F. ADDARII"

Viale Ercolani 4/2 — 40138 BOLOGNA, Italy. Phone 051 — 390417 Cable DEPCA — BOLOGNA

J. SÓS, T. GÁTI, L. CSALAY AND I. DÉSI

PATHOLOGY OF CIVILIZATION DISEASES

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