ACTA MEDICA

ACADEMIAE SCIENTIARUM HUNGARICAE

TOMUS XXXI FASCICULI 1—2

AKADÉMIAI KIADÓ, BUDAPEST

1974

ACTA MEDICA

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KIADÓHIVATAL: BUDAPEST V., ALKOTMÁNY UTCA 21.

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ACTA MEDICA

TOMUS 31

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (1—2), pp. 1—8 (1974)

INVOLVEMENT OF SPINAL MECHANISMS IN CCl_4-INDUCED ACUTE LIVER INJURY

By

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(Received October 30, 1972)

Division of the spinal cord in the thoracic region performed 2 hours before administration of CCl_4 was found to modify the typical hepatotoxic effect, as reflected by full preservation of lysosomal integrity, by the absence of hepatocellular necrosis and by less fatty degeneration, with no increase in the plasma-free fatty acid level. After chordotomy at the cervical level, fatty degeneration of the liver remained absent. Administration of CCl_4 4 days after chordotomy was followed by typical hepatotoxic manifestations. The results of the study fail to link up the modifications produced by the intervention with changes in body temperature or with factors involved by absorption and transport. It may be assumed that the spinal centres play a part in the production of liver injury.

Calvert and Brody were the first to draw attention to the involvement of the spinal centres in the production of CCl_4-induced liver injury. These authors, after having divided the spinal cord, studied the effect of CCl_4 on the mitochondrial enzyme system and on the microscopic structure of the liver, and found a significant modification of the liver injury (Calvert and Brody [4]; Brody et al. [3]). Other workers (Larson and Plaa [7, 9] Larson et al. [8]) attributed the modification in the CCl_4-induced liver injury consequent upon chordotomy to a fall in body temperature rather than to nervous mechanisms.

In view of the controversial nature of the subject, we intended to examine it by testing some parameters other than those studied by earlier workers, thus, the changes in lysosomal integrity, in lipid metabolism and in the microscopic structure of the liver, these being regarded as suitable indicators of hepatotoxic injury. Chordotomy was performed at the thoracic and cervical levels, and the modification of the CCl_4-induced abnormal reactions were studied in the first hours and a few days after the intervention. Body temperature was registered throughout the entire period and the fate of CCl_4 was followed up.

Material and methods

Home-bred albino rats of either sex weighing between 200 and 250 g, were studied. They were kept on granulated rat chow which was allowed ad libitum until the night preceding the experiment, then food was withdrawn with regard to the measurement of plasma-free fatty acids.

The spinal cord was divided at the level of the 4—5 thoracic or the 6—7 cervical vertebrae under 4 mg/100 g intraperitoneal pentobarbital anaesthesia. The wound was sprinkled

with penicillin. In the further course of the study the bladder was emptied daily by pressure on the vesical area. Protection against infections was provided by daily intramuscular injections of tetracyclin.

The chordotomized animals were kept at 18 to 20°C temperature. In one of the groups, rectal temperature was checked daily with a mercury thermometer.

CCl_4 was introduced through a stomach tube. After decapitation of the animals, their blood was collected and their liver excised for study.

Total, bound and free lysosomal acid phosphatase activity was estimated in liver homogenates by the method of De Duve et al. (De Duve et al. [6]; Wattiaux and De Duve [15]).

The lipid content of the liver was determined by gravimetry (Weil and Stetten [16]; Payne [11]). For the measurement of the plasma-free fatty acids, the method of Dole and Meinertz [5] was used.

CCl_4 concentration in the liver was estimated by the method of Recknagel and Litteria [13]. Histology of the liver was studied after haematoxylin-eosin staining by light microscopy.

Each group included 10 animals. The effect of CCl_4 administered in doses of 0.25 ml/100 g and of 0.5 ml/100 g by the oral route was studied with reference to normal controls. The animals were decapitated 24 hours after CCl_4 treatment. In one group the spinal cord was divided in the thoracic, in another group in the cervical region. CCl_4 was administered 2 hours after the intervention, and the parameters referred to above were examined 24 hours later. In a further group, CCl_4 was administered 4 days after the intervention, and the metabolic changes were studied 24 hours later.

A separate group was set up for the continuous registration of rectal temperature under the influence of CCl_4 administered 2 hours and 4 days after spinal cord division.

In additional experiments CCl_4 was administered orally 12 hours after chordotomy and the concentration of CCl_4 in the liver was estimated 1.5, 3, 6 and 9 hours later.

Results

a) *Lipid metabolism*

Twenty-four hours after CCl_4 administration, a 120% increase in the plasma-free fatty acid level was demonstrable, as compared with the normal controls ($p < 0.001$) (Fig. 1A). Twenty four hours after thoracic chordotomy, there was a 100% increase in the plasma free fatty acid level ($p < 0.01$). If the CCl_4 was administered 2 hours after chordotomy, its plasma concentration failed to rise above the value found in the chordotomized group ($p < 0.2$).

On the fifth day after thoracic chordotomy the plasma-free fatty acid level was normal (Fig. 2A). If the CCl_4 was administered 4 days after chordotomy, the typical increase was demonstrable at 24 hours; it corresponded to 70% as compared to the former group ($p < 0.01$).

As compared with the normal controls, CCl_4 produced a 2.5-fold increase in the lipid content of the liver by the end of 24 hours ($p < 0.01$) (Fig. 1B). 24 hours after thoracic chordotomy there was a 25% increase in the lipid content of the liver ($p < 0.05$). If the CCl_4 was administered 2 hours after the intervention, the rise in hepatic lipid content corresponded to 45% ($p < 0.05$) with reference to the chordotomy group, i.e. to 25 per cent less than in the case of CCl_4 administration to unoperated rats ($p < 0.05$). If the spinal cord was divided in the cervical region, CCl_4 administered 2 hours after this intervention failed to produce any increase in the total lipid content of the liver.

By the end of the fifth day after thoracic chordotomy, the lipid concentration in the liver returned to normal values (Fig. 2B). Administration of

CCl_4 on the fourth day after this intervention resulted in a more than 2.5-fold increase in lipid content of the liver ($p < 0.001$) by the end of 24 hours, which thus corresponded to the value produced in the unoperated animals. On doubling the dose of CCl_4 under these conditions, a further increase amounting to thrice the normal value ($p < 0.001$) was demonstrable. In other words, in these doses too, the toxic agent produced a similar effect in the operated and unoperated animals.

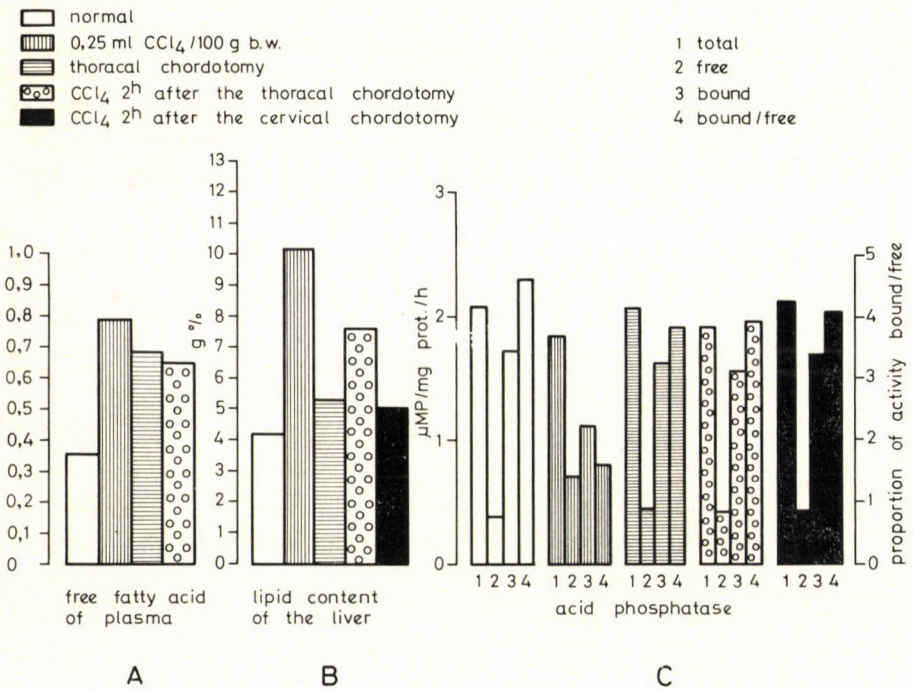

Fig. 1. Hepatotoxicity of CCl_4 administered 2 hours after chordotomy

b) *Lysosomal changes*

Twenty-four hours after thoracic chordotomy, lysosomal acid phosphatase activity was normal (Fig. 1C). In normal rats, CCl_4 produced an 80% increase in free activity ($p < 0.01$), a 35% decrease in bound activity ($p < 0.05$) and a 65% decrease of the ratio-bound activity/free activity ($p < 0.01$). Strikingly different values were noted if the spinal cord had been divided in the thoracic region 2 hours before the administration of CCl_4; as there was a 45% decrease in free activity ($p < 0.01$), a 35% increase in the bound activity ($p < 0.01$) and a 2.5-fold increase in the ratio-bound activity/free activity ($p < 0.0001$). If the spinal cord had been divided at the cervical level 2 hours before CCl_4 administration, the enzyme activities as well as their localization

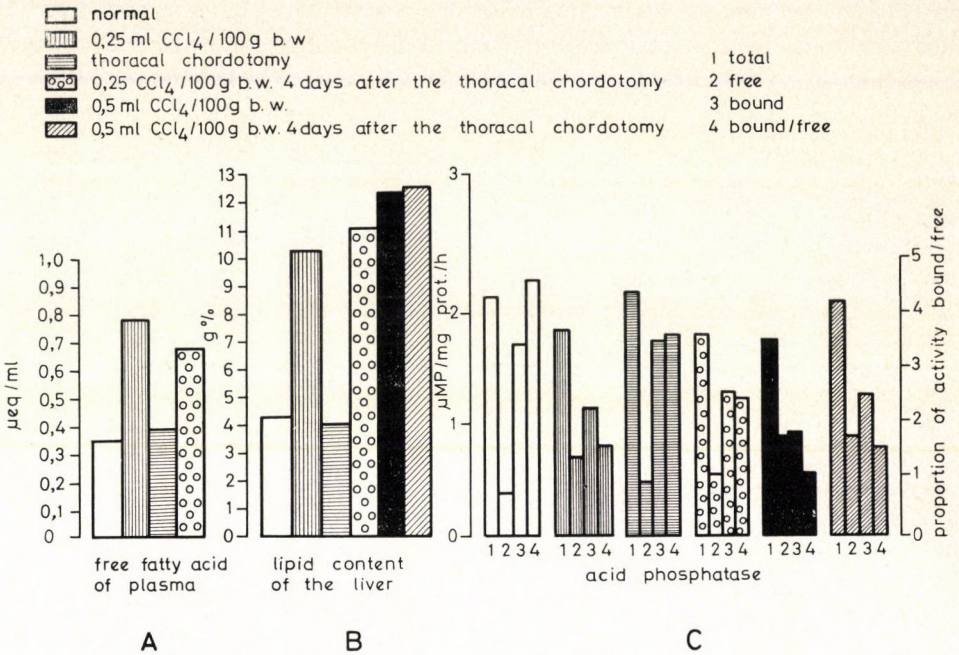

Fig. 2. Hepatotoxicity of CCl_4 administered 4 days after chordotomy

tended to shift still more to the values found in the normal controls. In particular, as compared with the CCl_4-treated normal animals, there was a 40% decrease in free activity ($p < 0.02$), a 50%-increase in bound activity ($p < < 0.01$), and a 3.5-fold increase in the ratio-bound activity/free activity ($p < < 0.001$). Administered under these conditions, CCl_4 thus leaves lysosomal integrity unaffected.

By the end of the fifth day after thoracic chordotomy, there was likewise no alteration in the lysosomal acid phosphatase activity (Fig. 2C). With reference to this group, CCl_4 administered 4 days after the intervention was found to produce the following changes by the end of 24 days: a 20% decrease in total activity, a 25% decrease in bound activity ($p < 0.05$) and a 35% decrease in the ratio-bound activity/free activity. Compared with the corresponding, CCl_4-treated controls, there was a 25% decrease in free activity ($p < 0.05$) and a 40% increase in the ratio-bound activity/free activity ($p < 0.05$). Though in this group the alteration in the localization of acid phosphatase was less marked, there was a definite fall in activity. Administration of CCl_4 in a dose of 0.5 ml/100 g 4 days after chordotomy resulted in an 80% increase of free activity ($p < 0.01$), a 30% one in bound activity ($p < 0.05$), and a 60% decrease in the ratio-bound activity/free activity ($p < 0.01$) by the end of 24 hours. As compared with the effect of 0.5 ml/100 g of CCl_4 in normal rats,

there was a 40% increase in bound activity as well as in the ratio-bound activity/free activity ($p < 0.05$). Thus, in this case, the alteration in the localization of lysosomal acid phosphatase was more marked, yet its total activity remained unchanged, it fell in the normal range.

c) *Microscopic appearance of the liver*

Twenty-four hours after oral administration of 0.25 ml/100 g of CCl_4, the histological appearance of the liver was marked by hydropic, vacuolar degeneration, pycnosis, dystrophy and acinocentral necrosis.

Twenty-four hours after division of the spinal cord in the thoracic region, pycnosis, nuclear changes and peripheral infiltration were found in the liver.

If the CCl_4 was administered 2 hours after thoracic chordotomy, the liver still exhibited signs of pycnosis and nuclear changes but no necrosis. The microscopic alterations were likewise confined to pycnosis and slight nuclear changes when chordotomy 2 hours before CCl_4 administration was performed at the cervical level.

By the end of the fifth day of the division of the spinal cord, the only histological change was a minor pycnosis. If, however, CCl_4 was administered 4 days after the intervention, in the liver hydropic and vacuolar degeneration, pycnosis and acinocentral degeneration ensued by the end of 24 hours.

Thus, CCl_4 produces no hepatocellular necrosis if administered within the first hours after chordotomy, but it produces definite hepatocellular necrosis if administered 4 days after the intervention.

d) *Body temperature*

By the end of 2 hours after chordotomy, there was a considerable fall in body temperature (approximately to 30°C), followed by a further decline (to 28°C) after the administration of CCl_4 (Table I).

Table I

Effect on rectal temperature of CCl_4 administered 2 hours after thoracic chordotomy

	Rectal temperature (°C)		
	2 hours after operation, before administration of CCl_4	3 hours after administration of CCl_4	20 hours after administration of CCl_4
1	30.2	29.2	38.4
2	31.8	32.0	28.6
3	31.0	30.8	32.2
4	28.0	31.4	28.0

By the end of the fourth day after chordotomy, before CCl_4 administration, the temperature rose to about 32°C but after administration it decreased again to about 30°C (Table II).

Table II

Rectal temperature during the first 5 days after thoracic chordotomy, with CCl_4 administered on 4th day

Day of intervention	Rectal temperature (°C)					
		1	2	3	4 (before CCl_4 administration)	5
1	31.6	34.0	34.2	33.4	33.0	31.2
2	32.0	30.0	29.8	31.6	31.8	29.2
3	32.0	32.0	32.6	33.2	32.6	28.8
4	32.8	32.2	33.2	32.8	32.4	30.4
5	33.6	33.2	33.8	34.2	33.2	30.2

e) *CCl_4 concentration in the liver*

In unoperated rats treated with CCl_4, the peak concentration in the liver was observed between the 3rd and 6th hour after administration (Table III). In chordotomized animals, high CCl_4 concentrations were demonstrable 1.5 hours after administration; the CCl_4 level remained high for 6 hours but declined thereafter.

Table III

Hepatic concentration of orally administered CCl_4 in unoperated and in chordotomized rats

Time after CCl_4 administration	CCl_4-content, $\mu g/g$ liver	
	Unoperated rats	Chordotomized rats
	160a	1240
1.5 hours	320	480
	200	1200
	1000	760
3 hours	960	1120
	840	880
	1160	1800
6 hours	1440	840
	920	440
	380	420
9 hours	560	540
	640	340

Discussion

The first hours after division of the spinal cord are marked by spinal shock and by paralysis of the spinal centres inferior to the level of division (MORIN [10]). These centres may well affect the production of liver injury, and this accounts for the finding that if chordotomy is performed 2 hours before CCl_4 administration, the characteristic liver injury remains absent.

In the course of time, the spinal centres gradually resume their function. This is probably the reason why the hepatotoxic reaction proceeds unhindered if the toxic agent is administered as late as four days after chordotomy, although the lesion of the long tracts has remained unchanged. It is therefore evident that the failure of CCl_4 to produce hepatotoxic damage in the early phase after division of the cord is due to a paralysis of the spinal function rather than to interruption of the long tracts.

Minor alterations in lipid metabolism were demonstrable 24 hours after chordotomy in the control animals as well. One of the changes was a slight rise in the total lipid concentration of the liver, due in all probability to the fall in body temperature. BRAUER et al. [2], studying the influence of hypothermia on liver function, have found a slight fatty infiltration of the organ as a result of the fall in body temperature. The other remarkable alteration observed in the present study was a rise in the plasma-free fatty acid level 24 hours after chordotomy. Considering, however, that the animals had no food at that time, the elevated plasma fatty acid level may be attributed to fasting which has been found to act in this way independently of any nervous mechanism (PINTER and PETTEC [12].

The degree of fatty infiltration caused by CCl_4 in the liver depends on the level at which the spinal cord has been divided 2 hours before. The cause of this is probably that in the case of thoracic chordotomy the innervation of a large mass of fatty tissue is spared so that it may provide the necessary amount of fatty acids for fatty degeneration. On the other hand, division of the spinal cord at the cervical level affects the nervous supply of practically the whole fatty tissue, thus blocking the mobilization of fatty acids necessary for the infiltration. As a result, fatty degeneration remains absent. Chordotomy in the thoracic as well as in the cervical region takes place above the level of hepatic innervation. Therefore, eventual hepatic mechanisms do not seem to account for the changes in lipid metabolism, the less so, as the part played by the sympathetic system in the regulation of the lipolytic processes is a long-established fact (BEZNÁK and HASCH) [1], and the primary sympathetic centre of this mechanism is situated in the spinal cord.

Our results indicate that after chordotomy, absorption of CCl_4 from the intestine proceeds normally and its transport to the liver is even more rapid than in unoperated rats. These factors having remained unaffected, any in-

volvement of absorption or transport factors in the preventive effect of chordotomy on hepatotoxic lesions can safely be ruled out. Since the toxic agent reaches the liver, any possible part played by haemodynamic factors in the prevention of hepatotoxic lesions can also be excluded, since in the case of a haemodynamic involvement, the blood supply to the liver would have had to increase after chordotomy. In fact, the opposite was the case. On the evidence of haemodynamic studies subsequent to chordotomy (TAKÁCS et al. [14]), cardiac output decreases and its organ fractions are not modified to any significant degree.

The hepatotoxicity of CCl_4 asserted itself at the same body temperature 2 hours as well as 4 days after the intervention. Yet, no injury developed if the CCl_4 had been administered 2 hours after chordotomy, while it developed when the poison had been administered 4 days after division of the cord. This pleads against any possible involvement of body temperature in the modifications brought about by chordotomy.

In the light of these facts it seems reasonable to attribute the modification of CCl_4-induced liver injury demonstrable within the very first hours after chordotomy to a transitory paralysis of the spinal centres and to rule out any possible involvement of hypothermia or of other factors affecting absorption and transport. The question whether this permissive mechanism acts through direct neural pathways or whether it involves some intermediary factors dependent on the spinal centres, has to be clarified by further studies.

REFERENCES

1. BEZNÁK, A. B. J., HASCH, Z.: Quart. J. exp. Physiol., **27,** 1 (1937).
2. BRAUER, R. W., HOLLOWAY, R. J., KREBS, J. S., LEONG, G. F., CARROL, H. W.: Ann. N. Y. Acad. Sci. **80,** 395 (1959).
3. BRODY, T. M., CALVERT, D. N., SCHEIDER, A. F.: J. Pharmacol. exp. Ther. **131,** 341 (1961).
4. CALVERT, D. N., BRODY, T. M.: Amer. J. Physiol. **198,** 669 (1960).
5. DOLE V. P., MEINERTZ, H.: J. Biol. Chem., **235,** 2595 (1960)
6. DUVE, CH. DE, PRESSMANN, B. C., GIANETTO, R., WATTIAUX, R., APPELMANS, F.: Biochem. J., **60,** 604 (1955).
7. LARSON, R. E., PLAA, G. L.: Experientia (Basel) **19,** 604 (1963).
8. LARSON, R. E., PLAA, G. L., GREWS, L. M.: Toxicol. appl. Pharmacol. **6,** 154 (1964).
9. LARSON, R. E., PLAA, G. L.: J. Pharmacol. exp. Ther. **147,** 104 (1965).
10. MORIN, G.: Physiologie du système nerveux central. Masson et Cie, Paris 1955.
11. PAYNE, R. W.: Endocrinology **45,** 305 (1949).
12. PINTER, E. J., PETTEC, C. J.: J. clin. Endocr. **27,** 1441 (1967).
13. RECKNAGEL, R. O., LITTERIA, M.: Amer. J. Path. **36,** 521 (1960).
14. TAKÁCS, L., DEBRECZENI, L. A., ALBERT, K.: Acta physiol. Acad. Sci. hung. **32,** 263 (1967).
15. WATTIAUX, R., DUVE, CH. DE: Biochem. J. **63,** 606 (1956).
16. WEIL, R., STETTEN, D.: J. biol. Chem. **168,** 129 (1947).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (1—2), pp. 9—13 (1974)

EXPERIMENTAL PRODUCTION OF ANTIBODIES AGAINST CATARACTOUS HUMAN LENS

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(Received February 15, 1973)

Rabbits and guinea-pigs were immunized with human cataractous lens homogenates, half of the animals capsulated, and half with decapsulated lens. In the rabbits treated with decapsulated lens-homogenate, immunoelectrophoresis yielded 5, in those treated with capsulated lenses, only 2, precipitation bands. No immune body to capsulated lens was demonstrable in the guinea-pigs, while in those immunized with decapsulated lens-homogenate, electrophoresis yielded one precipitation band. Thus, the capsule of the lens seems to be involved in immune processes.

Immunological studies of the proteins of the crystalline lens date back to the first decade of the century when the sensitizing properties of heterogeneous lens proteins had been demonstrated by UHLENHUTH [20] and confirmed in subsequent animal experiments [1, 13, 18, 19]. The lens being well-known for its weak antigenic character, in most of these experiments different adjuvants were applied for enhancing the production of antibodies. At present, the most extensively used type is Freund's complete adjuvant which contains, in addition to mineral oil and to aquaphor, heat-killed Mycobacterium tuberculosis.

Since the description by VERHOEFF and LEMOINE [21] of the endophthalmitis phacoanaphylactica, the phaco-antigens have been widely studied, although the pathogenesis of the syndrome has remained obscure.

With the progress in immunological techniques it has become possible to re-examine the antigenic components of the lens. After immunization of rabbits with human normal or cataractous lenses, 5 to 9 precipitation bands were demonstrated by immunoelectrophoresis or immunodiffusion [5, 6, 7, 8, 9, 10, 11, 16, 23, 24]. These bands seem to fall into 3 major fractions, probably in agreement with the alpha, beta and gamma crystallins.

In the present study too, human lens served as antigen for the production of antibodies in rabbits and guinea-pigs. In earlier experiments, the lens was either decapsulated or homogenized together with its capsule. This factor has, however, been disregarded by the authors when evaluating their results. In order to establish whether this point was of any relevance, we used extracts of decapsulated lenses in half of the animals, whereas for the immunization of the other half, extracts of lenses with the capsules left in place were used.

Material and methods

Antigens. The cataractous human lenses were placed immediately after their extraction into Coca's solution as proposed by MARTINA[12] (0.5% NaCl, 0.4% phenol, 0.25% $NaHCO_3$). Four to 8 lenses were collected in 20 ml and stored in the deep-freezer until processing. Before use the lenses were homogenized with teflon-glass, centrifuged at 10 000 r. p. m. at 4°C for 10 min. Protein was measured in the supernatant by Kjeldahl's method. Half of the lenses were homogenized together with their capsule, the other half was decapsulated immediately after extraction.

Immunization. Four chinchilla-rabbits weighing between 3.0 and 3.5 kg and 4 guinea-pigs weighing between 250 and 300 g were used. Two rabbits were immunized with capsulated, two with decapsulated lens extract, 1 ml extract being administered intramuscularly twice weekly over ten weeks, changing the sites of injection every time. The extract of capsulated lenses contained approximately 15 mg, that of the decapsulated lenses 10 mg protein per ml. Irrespective of the type of extract, the animals were given 1 ml of Freund's complete adjuvant intramuscularly prior to the study and 4 weeks later, thus on two occasions altogether. Eight days after the last injection the animals were killed by exsanguination.

The guinea-pigs likewise received the capsulated and the decapsulated lens extracts in two groups, twice a week over 10 weeks. Freund's complete adjuvant was also administered, in the same manner as to the rabbits. One of the animals immunized with the extract of capsulated lenses died from an abscess 8 weeks later. The remaining 3 animals were exsanguinated 8 days after the last injection.

Immunoelectrophoresis. SCHEIDEGGER's microtechnique [17] was used, 4 ml 1% Difco Noble agar solution was spread on each plate, the antiserum was run for 3 hours, washed, dried and stained with fuchsin.

Results

On the evidence of the precipitation bands, the cataractous human lens proved antigenic to rabbits and guinea-pigs. Extracts of capsulated lenses gave patterns different from those of the decapsulated ones. The cataractous lenses yielded at least 5 antigen components; their precipitation bands were distinctly separated at adequate dilutions (Fig. 1). On the other hand, the

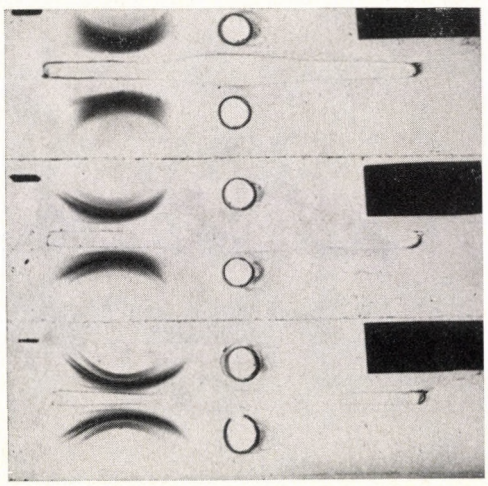

Fig. 1. Antibodies raised in rabbits against human decapsulated lens. Dilutions: top, 1 : 1; middle 1 : 2; bottom 1 : 4

antibody response to capsulated lenses was less intensive, as only 2 precipitation bands were obtained (Fig. 2).

In guinea-pigs, the antigenicity of the capsulated lens extract proved too weak to form detectable antibodies despite administration of Freund's adjuvant. The decapsulated lens extract yielded one precipitation band (Fig. 3).

During the entire study, the eyes of the animals were kept under close observation; they displayed no inflammatory reaction.

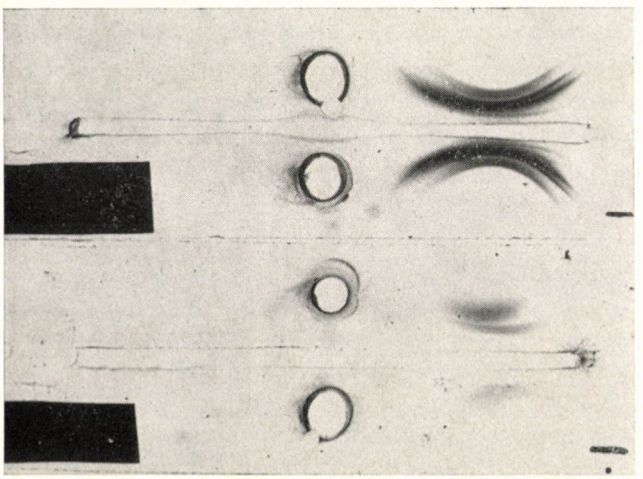

Fig. 2. Antibody response in rabbits to capsulated lens extract (top) is less intensive than that to decapsulated lens extract (bottom). Dilution 1 : 4

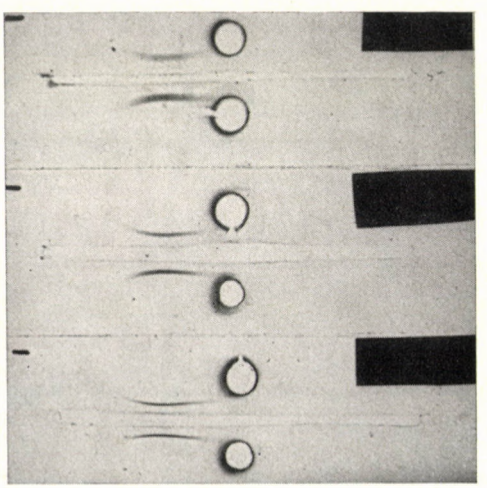

Fig. 3. Antibody response in guinea-pigs is confined to decapsulated lens extract. Dilutions: top 1 : 1; middle 1 : 2; bottom 1 : 4

Discussion

The results showed that antibody production is readily elicited in heterogeneous species, and, further, that the presence of the capsule is by no means indifferent. It has to be assumed that the capsule plays some hitherto unidentified role in the immune process.

According to WIROTSKO and HALBERT [22], the capsule of the lens differs in respect of antigenicity from other soluble phacoproteins, presumably as a result of its chemical structure. DISCHE and ZELMENIS [3] found the capsular proteins to be reminiscent of collagens in respect of their biological and chemical properties.

Earlier, the capsule was regarded mainly as a barrier to the passage of intracapsular proteins into the aqueous humour, maintaining the chemical stability of these soluble proteins [14].

We have thus to assume that the presence of the capsule affects intensity of the immune response, since our procedure has been identical all throughout, the only difference having been the presence or absence of the capsule. At any rate, although the capsulated lens extract contained more protein than that of decapsulated lenses (15 mg/ml, and 10 mg/ml, respectively), the immune response to the latter was none the less more intensive than to the former in the rabbit and the guinea-pig alike.

It would seem obvious to connect this phenomenon with post-mortem enzyme activity, this being the highest in the capsule [4]. The finding that an injury of the capsule is followed by a significant increase in enzyme activity of the aqueous humour [15] is consistent with this claim.

ZWAAN [25] who identified alpha and beta crystallins in the aqueous humour in the absence of any capsular lesion, points to post-mortem activities and to certain problems of processing as the source of false results. It is conceivable, on the other hand, that the capsule possesses some inhibitory factor which, under particular circumstances, might be able to neutralize the antigenicity of the soluble proteins. Conjectures of this kind have been voiced earlier in the literature. In fact, the possibility that on release from the lens, alpha crystallin might be neutralized by beta and gamma crystallins, has been raised by BURKY and WOODS [2]. Studies with isolated lens factors have certainly the advantage of greater accuracy, but immunization with total capsulated or decapsulated lens-extracts is expected to give a closer insight into the reactions in vivo.

Further investigations would be desirable in order to throw light on the pathogenesis of phacogenic uveitis, it being all too well known that the residue left in place after cataract extraction may contain capsular and non-capsular elements alike.

Clarification of the part played by the capsule awaits further studies.

Acknowledgements

Our thanks are due to Professor B. CSABA, Institute of Pathophysiology, for his valuable aid in the design and execution of the present experiments; and to Miss A. NAGY for her technical assistance.

REFERENCES

1. BURKY, E. L.: Arch. Ophthal. (Paris) **12,** 536 (1934).
2. BURKY, E. L. and WOODS, A. C.: Arch. Ophthal. **6,** 548 (1931).
3. DISCHE, Z. and ZELMENIS, G.: Invest. Ophthal. **4,** 174 (1965).
4. FRIEDBURG, D.: Albrecht v. Graefes Arch. Ophthal. **175,** 46 (1968).
5. HALBERT, S. P. and FITZGERALD, P.: Amer. J. Ophthal. **46,** 187 (1958).
6. HALBERT, S. P., LOCATCHER-KHTORAZO, D., SWICK, L., WITMER, R., SEEGAL, B. and FITZGERALD, P.: J. exp. Med., **105,** 439 (1957).
7. LEURE DU PREE, A., LITTLE, J. and LANGMAN, J.: Arch. Ophthal. **72,** 660 (1964).
8. LITTLE, I., IKEDA, A., ZWAAN, J. and LANGMAN, J.: Exp. Eye Res. **4,** 187 (1965).
9. LITTLE, J. and LANGMAN, J.: Arch. Ophthal. **72,** 820 (1964).
10. MAISEL, H. and GOODMAN, M.: Invest. Ophthal. **4,** 129 (1966).
11. MANSKI, W., AUERBACH, T. and HALBERT, S. P.: Amer. J. Ophthal. **50,** 315 (1960).
12. MARTINA, F.: Ophthalmologica (Basel) **139,** 84 (1960).
13. MÜLLER, H.: Albrecht v. Graefes Arch. Ophthal. **153,** 1 (1952).
14. ORBÁN, T. and VELEZNAY, ZS.: Szemészet **100,** 71 (1963).
15. OTTO, J. and HAHNEL, R.: Klin. Mbl. Augenheilk. **137,** 286 (1960).
16. RAO, S. S., KULKARNI, M. E., COOPER, S. N. and RADHAKRISHNAN, M. R.: Brit. J. Ophthal. **39,** 163 (1955).
17. SCHEIDEGGER, J. J.: Sem. Hop. Paris **32,** 2119 (1956).
18. SCOBEE, R. G. and SLAUGHTER, H. C.: Amer. J. Ophthal. **27,** 49 (1944).
19. SWIFT, H. F. and SCHULTZ, M. P.: J. exp. Med., **63,** 703 (1936).
20. UHLENHUTH, P. T.: Zur Lehre von der Unterscheidung verschiedener Eiweißarten mit Hilfe spezifischer Sera. Festschrift zum 60. Geburtstag von Robert Koch. G. Fischer, Jena 1903. p. 49.
21. VERHOEFF, F. H. and LEMOINE, A. N.: Trans. int. Congress of Ophthalmology **1,** 234 (1922).
22. WIROTSKO, E. and HALBERT, S. P.: Autoimmune phenomena in the eye. In: Textbook of immunopathology. Ed.: Miescher, P. A. and Müller-Eberhardt. H. J. Grune and Stratton, New York 1969. pp. 624—640.
23. WITMER, R.: Arch. Ophthal. **53,** 871 (1955).
24. WITMER, R. und BÜHLER, E.: Albrecht v. Graefes Arch. Ophthal., **162,** 193 (1960).
25. ZWAAN, J.: Immunochemical analyses of the eye lens during development. Rototype, Amsterdam 1963.

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (1—2), pp. 15—24 (1974)

ASSESSMENT OF SPATIAL VELOCITY OF VCG IN INTRAVENTRICULAR CONDUCTION DISTURBANCES

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(Received 28 June, 1973)

The spatial velocity of the vectorcardiogram has been derived from the components x, y and z of the Frank lead system by means of analogue computers and recorded in a scalar form on a direct-writer electrocardiograph.

The spatial velocity curves were analyzed for quantitative relationships and configuration, the normal features having been studied in 30 individuals without any evidence of heart disease. In 28 patients with left bundle-branch block, a decrease in velocity was demonstrable in the middle portion of the QRS complex. The sV record permits to differentiate the uncomplicated cases of left bundle-branch block from those associated with myocardial infarction. In 19 patients with right bundle-branch block, a considerable decrease in velocity was observed in the second portion of the QRS complex. The sV ECG in left anterior hemiblock showed two well-defined patterns. In the 11 cases of right or left ventricular pacing the high-velocity spike was followed by a considerable decrease in velocity, in accordance with the retrograde spread of impulse. The sV ECG is claimed to provide the most reliable information on the velocity of conduction, on the site of its decrease and on its extent in the case of intraventricular conduction disturbances.

A complex study of the heart's bioelectric activity is no longer conceivable without the use of computers. The new spatial parameters derived from corrected orthogonal leads (spatial magnitude of vector, azimuth and elevation angles) reflect the electric events more reliably and more illustratively than do the conventional ECG leads. (For a survey, see ANTALÓCZY [1]).

The spatial velocity curve offers further mathematical information contained in the vectorcardiogram and permits an exact analysis of the velocity of the spread of impulse.

The spatial velocity curve represents the changes in the velocity of the spatial vector in a scalar form. Spatial velocity (sV) can be calculated from the formula

$$sV = \sqrt{\left(\frac{dx}{dt}\right)^2 + \left(\frac{dy}{dt}\right)^2 + \left(\frac{dz}{dt}\right)^2}$$

where x, y and z represent the instantaneous vectors of the three corrected orthogonal Frank leads. The square root of the summed squares of the first derivatives gives the actual value of the velocity vector.

Material and methods

Spatial velocity of the VCG was estimated by an analogue computer of our own construction. The circuit representing the sV ECG was attached to an EMG Biokomb 5-type polygraph adapted for registration of Frank leads [4]. The direct-writing apparatus registers the sV tracing simultaneously with the components x, y and z. The paper speed was 50 mm/sec and 100 mm/sec.

Spatial velocity is represented as a positive curve. The measured amplitudes give the values for the individual actual vectors directly in mV/sec. Technical details of the apparatus have been published earlier [5].

The present study was confined to the definition of the spatial velocity of QRS. The tracings were analyzed for their quantitative relationships as well as for their configuration.

The curves were divided into 10 equal parts. The means S. D. of the n/10 actual vectors and the fiducial limits were determined for each individual group. Significance was calculated by means of Student's two-sample t test.

Analysis of the configuration of the sV curve was performed by determining its characteristic maximum and minimum values, their means and scatter and the 95% fiducial limits being assuming a normal distribution, and the average timing of these parameters.

Results

Normal control group

This group included 30 healthy subjects from 18 to 40 years of age with no evidence of any ECG or VCG abnormality.

The sV ECG of QRS consists normally of two main deflections preceded or followed by a minor component.

The normal curve is M-shaped (Fig. 1). Within this pattern, two main types may be distinguished according to the height at which the dip between the two main deflections is located.

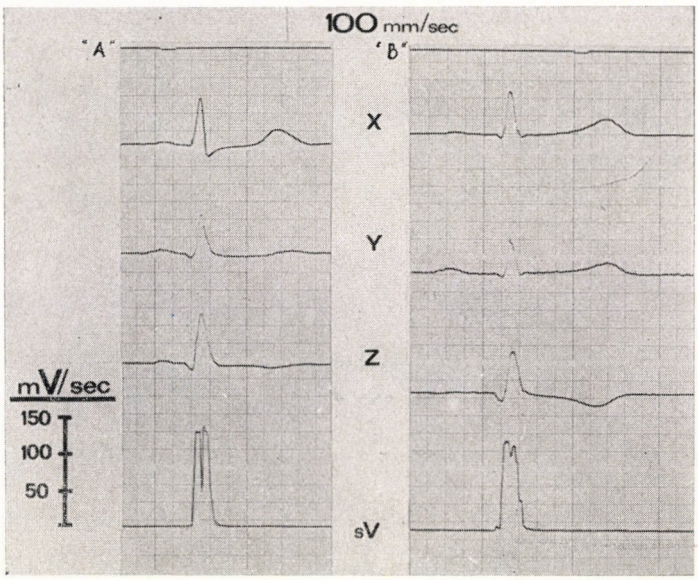

Fig. 1. Normal type A and B sV curve

Three characteristic points have been selected on the normal sV ECG: the first and second maximum points (ϱ_1 and ϱ_2) and the minimum (D), falling between the two peaks (Fig. 2).

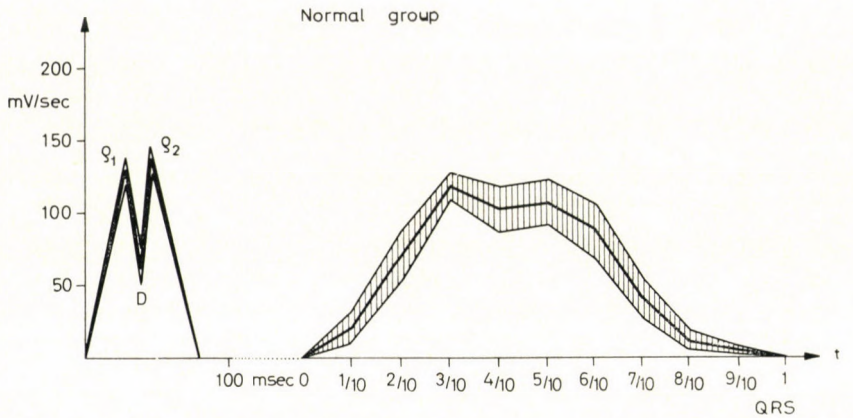

Fig. 2. Statistical analysis of normal sV curve. Left side: evaluation of configuration. Right side: analysis on the basis of n/10 division

Features of the normal tracing

	ϱ_1	D	ϱ_2
$\overline{X}$	125.9	90.0	133.4 mV/sec
95% fiducial limit	±3.6	±11.2	±4.4 mV/sec
Average timing	27.7	36.8	44.1 msec

The QRS divided into 10 equal parts failed to display the finer details, in particular the D-minimum on the normal tracing, probably because it falls between two samplings.

Features of the n/10 sV actual vector

	1/10	2/10	3/10	4/10	5/10	6/10	7/10	8/10	9/10	QRS
X	18.6	69.8	114.5	100.9	104.8	86.5	40.2	10.1	3.4	0
95% fiducial limit	±10.5	±16.7	±8.4	±12.8	±15.6	±16.9	12.3	±2.5	±0.7	0

Left bundle-branch block. The sV curve of 28 patients has been analyzed; the left bundle-branch block was uncomplicated in 15, and associated with myocardial infarction in 13 cases.

On the sV of uncomplicated left bundle-branch block, an initial and a terminal peak (ϱ_1 and ϱ_2) could be distinguished. In the middle section of the QRS, between D_1 and D_2, conduction slows down (Fig. 3).

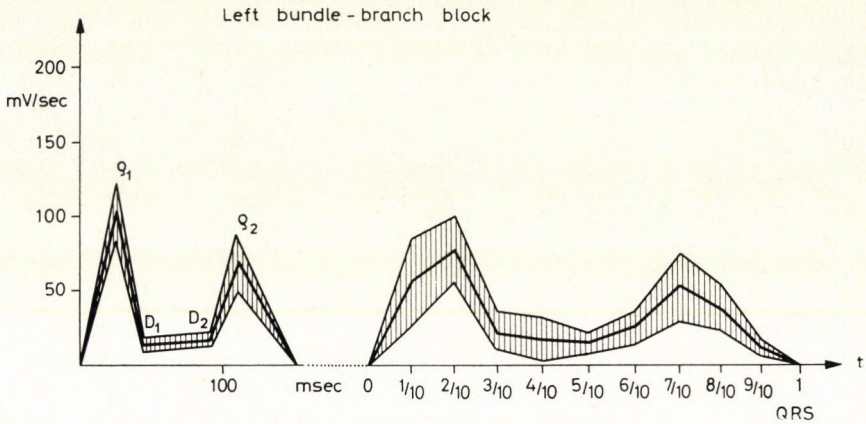

Fig. 3. sV ECG of left bundle-branch block

Features of the sV curve

	ϱ_1	D_1	D_2	ϱ_2
$\bar{X}$	104.3	9.5	13.0	70.0 mV/sec
95% fiducial limit	±19.3	±4.0	±4.1	±20.3 mV/sec
Average timing	25.3	42.3	93.0	109.0 msec

Average width of QRS is 147 msec. Maximum velocity is attained in the initial part of the QRS. Slowing of conduction occurs between 42.3 and 93.0 msec. Velocity increases again terminally, without, however, attaining that of the initial phase ($\varrho_1 > \varrho_2$).

The result obtained by quantitative analysis on the n/10-point basis agreed well with the assessment of configuration.

In left bundle-branch block associated with myocardial infarction, configuration of the sV ECG was similar as in the uncomplicated form. There were two peaks (ϱ_1 and ϱ_2). In the middle portion, between D_1 and D_2, velocity diminishes. Lower amplitudes and a more marked decrease in velocity in the terminal portion were found in the cases associated with myocardial infarction.

The width of the curve amounted to 140.8 msec on the average. Slowing of conduction fell between 43.5 and 80 msec. In the n/10 division the definite slowing of conduction in the terminal section is clearly seen. The difference in magnitude of the QRS sV vectors 7/10 and 8/10 between uncomplicated left

Features of the sV ECG

	ϱ_1	D_1	D_2	ϱ_2
$\overline{X}$	96.9	8.8	7.2	45.8 mV/sec
95% fiducial limit	±23.2	±3.2	±2.5	±21.9mV/sec
Average timing	24.6	43.5	80.0	95.4 msec

bundle-branch block and bundle-branch block associated with myocardial infarction was significant statistically ($p < 0.05$).

Right bundle-branch block. The sVECG was studied in a total of 19 patients with right bundle-branch block; 14 cases were uncomplicated and 5 associated with myocardial infarction.

In simple block the first half of the sV curve showed two peaks (ϱ_1 and ϱ_2) and a minimum (D). In the second portion of the curve there was a marked decrease in velocity (its beginning is marked by x). The terminal portion showed a slight increase in velocity (8) (Fig. 4).

Typical features

	ϱ_1	D	ϱ_2	x	ϱ
$\overline{X}$	127.1	24.9	126.4	4.1	13.4 mV/sec
95% fiducial limit	±24.6	±15.2	±32.8	±1.9	±9.0 mV/sec
Average timing	30.3	41.4	50.4	66.4	111.4 msec

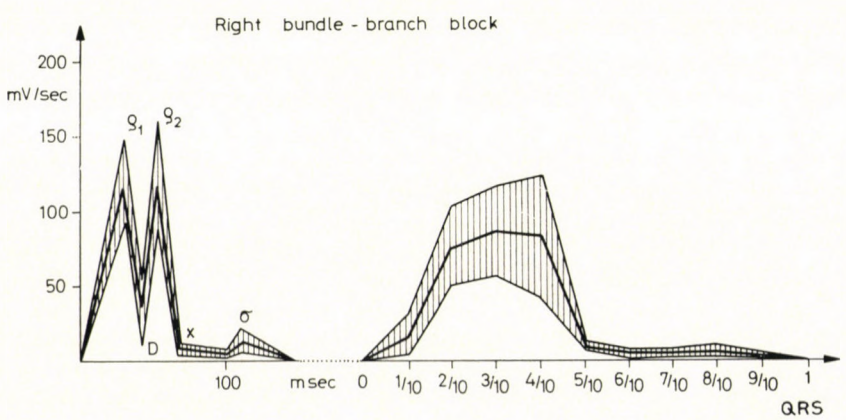

Fig. 4. sV ECG of right bundle-branch block

Mean width of the curve was 141.8 msec, the decrease in velocity began at 66.4 msec. Its terminal attained only 10% of the peak. The n/10-point division failed to show those details of configuration. Slowing of QRS began at the actual vector 5/10.

In right bundle-branch block associated with myocardial infarction the sV curve was reminiscent in configuration of that found in simple right bundle-branch block. Mean width of the complex measured 127 msec. Slowing of conduction could be localized at 65 msec. The n/10 point system and configuration analysis gave comparable results. A statistically significant difference was found between the two groups only in the magnitude of the QRS sV 5/10.

Left anterior hemiblock. The sV curve has been studied in 17 patients. The diagnosis of left anterior hemiblock was based on the usual criteria. In 6 cases left anterior hemiblock was associated with myocardial infarction.

Configuration analysis allowed to divide the curves into two types.

Type A (6 cases)

The first peak is lower than the second ($\varrho_1 < \varrho_2$) (Fig. 5), mean width was 91.7 msec.

Typical features

	ϱ_1	D	ϱ_2	Y
$\bar{X}$	97.5	3.2	163.3	4.3 mV/sec
95% fiducial limit	±48.5	±2.6	±10.8	±3.9 mV/sec
Average timing	32.5	39.2	50.0	62.5 msec

Type B (5 cases)

The first peak is higher than the second ($\varrho_1 > \varrho_2$) (Fig. 6), mean width was 118 msec.

Typical features

	ϱ_1	D	ϱ_2	Y
$\bar{X}$	150	46	119	14 mV/sec
95% fiducial limit	±23.6	±5.3	±30	±15.5 mV/sec
Average timing	27	43	70	84 msec

Slowing of conduction fell between 84 and 118 msec.

Analysis on the basis of the n/10 division and on the basis of configuration yielded comparable results.

Between groups A and B there was a significant difference in respect of the time intervals 2/10, 3/10, 4/10, 5/10, 8/10 and 9/10. The most conspicuous

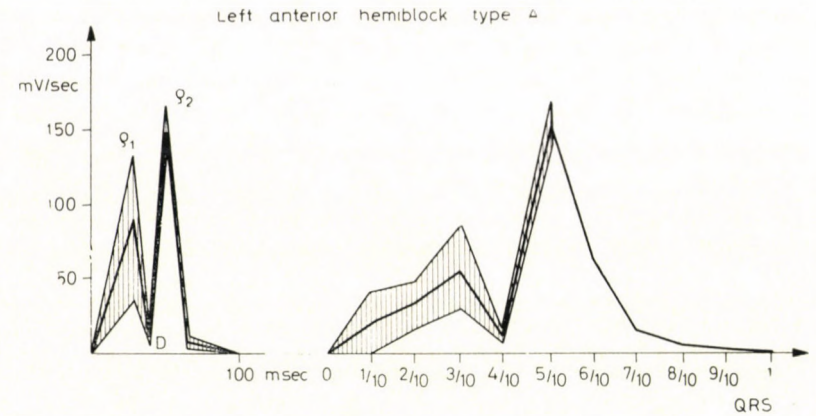

Fig. 5. sV ECG of left anterior hemiblock, type A

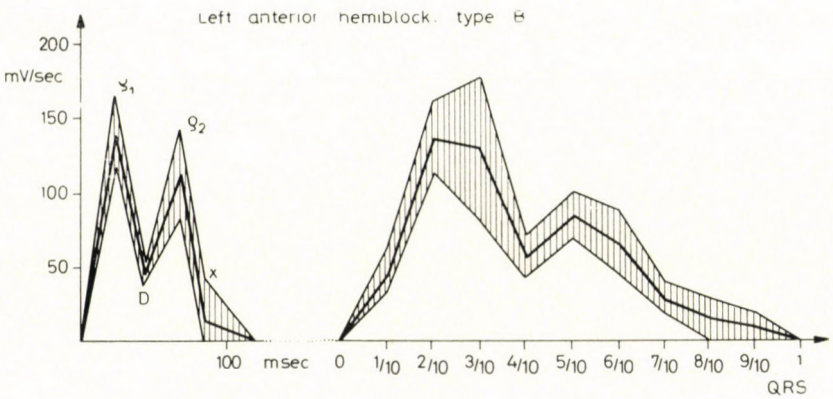

Fig. 6. sV ECG of left anterior hemiblock, type B

difference between uncomplicated left anterior hemiblock and its form associated with myocardial infarction lies in a definite decrease in the amplitudes.

Between type A left anterior hemiblock and its form associated with myocardial infarction, there was a significant difference ($p < 0.05$) in the amplitudes in *sections* 4/10 and 5/10.

In right bundle-branch block combined with left anterior hemiblock, the sV was studied in 6 cases.

The pattern was reminiscent in its configuration of that found in uncomplicated right bundle-branch block, displaying two peaks (ϱ_1 and ϱ_2) and a minimum (D) between them. The beginning of decrease in conduction velocity is marked *x*. Mean duration of the complex was 133.3 sec.

Typical features

	ϱ_1	D	ϱ_2	x
$\overline{X}$	103.3	9.0	11.7	7.5 mV/sec
95% fiducial limit	±26.0	±4.9	±52.4	±6.4 mV/sec
Average timing	25.8	38.3	54.2	67.5 msec

Pacemaker stimulation. The sV was studied in 11 patients with right or left ventricular pacing; 5 of these patients had experienced myocardial infarction.

The sV curve begins with a high-amplitude component corresponding to the pacemaker spike, its amplitude exceeding 200 mV/sec and its duration being 2 msec in the majority of cases. This is followed, in accordance with the retrograde spread of ventricular stimulation, by a considerable decrease in velocity (Fig. 7). The extent of slowing and, hence, the configuration of

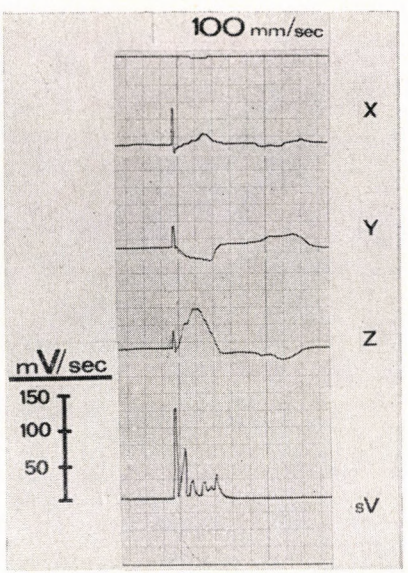

Fig. 7. sV ECG in case of pacemaker stimulation

the tracing, show wide individual variations, therefore no statistical analysis was performed.

With left ventricular pacing, the sV was consistent with the pattern of right ventricular stimulation.

Discussion

The spatial velocity curve described by HELLERSTEIN and HAMLIN [3] permits a reliable definition of the velocity of the activation process; it can be determined by analogue [6, 8] or digital [2, 10] computers. The analogue computers are preferable as they allow an evaluation of the configuration. Registration by a direct-writing apparatus is simple and expeditious. Statistical evaluation by the two methods has provided a reliable basis for the definition of the sV patterns of individual ECG syndromes.

Japanese authors [7, 9] have studied the sV ECG in myocardial infarction, ventricular hypertrophy and bundle-branch block. Among all the electrocardiologic procedures, the sV curve has been found to provide the most reliable information in the case of intraventricular conduction defects.

A decrease in conduction velocity is displayed on the ECG in the form of notching, on the VCG as a condensation of the time-darts of the Lissajous-loop. The sV ECG provides for quantitative analysis, in other words, it gives the absolute magnitudes of the individual actual vectors of velocity and permits an accurate localization of the timing of conduction defects, i.e. of the decrease in velocity.

In the present study conduction defects of different types have been examined for their sV ECG pattern. In left bundle-branch block the sV was found to decrease considerably in the middle portion and to increase again terminally. A right bundle-branch block is characterized by an uniform slowing in the second portion of the QRS.

To our best knowledge, the sV curve has not been studied before in connection with the different types of bundle-branch block and with pacemaker-stimulation.

The sV ECG allowed to discern two types of left anterior hemiblock, viz. type A characterized by a narrow QRS, with the second peak being taller than the first; and type B with a QRS of a mean width greater than in the first type and with its first peak taller than the second. The deep S found in lead V_5 of the ECG in these patients is believed to be indicative of a latent right ventricular conduction defect.

The sV ECG in bifascicular block is similar to that found in right bundle-branch block.

In case of pacemaker-stimulation, the sV ECG is marked by a high velocity spike, followed, in accordance with retrograde myocardial conduction, by a decrease in conduction to an individually variable extent.

The sV ECG helps to identify myocardial infarction in the case of intraventricular conduction defects. In left bundle-branch block, myocardial infarction results in a further, in the first place terminal, decrease in velocity, the difference between uncomplicated left bundle-branch block and its form

associated with myocardial infarction having been found significant in this respect. The sV ECG has thus proved a new helpful tool in the differentiation of two groups.

REFERENCES

1. Antalóczy, Z.: A szív elektromos működésének vizsgálata. Medicina, Budapest 1972
2. Antalóczy, Z., Strommer, M., Regős, L.: Triaxicardiometric quantitative vector analysis in myocardial infarction. Meeting of Hungarian Cardiologists Association. Balatonfüred 1972.
3. Hellerstein, H. K., Hamlin, R.: Amer. J. Cardiol., **6,** 1049 (1960).
4. Kenedi, P., Kamarás, I.: Orv. és Techn. **10,** 4 (1972).
5. Kenedi P., Müller, Gy., Székely, Á.: Cardiol. hung., **1,** 48 (1972).
6. Mori, H.: Jap. Circulat. J., **32,** 149 (1968).
7. Mori, H.: Jap. Circulat. J., **35,** 791 (1971).
8. Sano, T., Suzuki, F., Hiroki, T., Sawanobori, T.: Jap. Heart. J., **9,** 64 (1968).
9. Sano, T., Suzuki, F., Hiroki, T., Sato, S.: Proc. Jap. Acad., **43,** 812 (1967).
10. Yano, K., Pipberger, H. V.: Circulation **29,** 107 (1964).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (1—2), pp. 25—29 (1974)

URINARY EXCRETION OF ACIDIC GLYCOSAMINOGLYCANS IN BILHARZIASIS

By

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(Received, August 1, 1973)

Acidic glycosaminoglycans (AGAG) in urine have been estimated in 15 normal subjects and 54 bilharziasis patients. The bilharziasis patients were divided into four groups according to the severity and progress of the disease: (i) active bilharziasis infection; (ii) clinical hepatosplenomegaly; (iii) clinical hepatosplenomegaly plus ascites; (iv) hepatosplenomegaly plus ascites and oedema.

Urinary AGAG excretion was significantly elevated in all the patients with the exception of those in group (iv). Excretion of heparitin sulfate was increased in every patient. Thus connective tissue metabolism is affected by bilharzial infection.

Introduction

An increase in the urinary AGAG excretion has been described in Hurler's syndrome [1], Marfan's syndrome [2], the Morquio—Ullrich syndrome [3], inflammatory conditions of the connective tissue such as rheumatoid arthritis [4] and systemic lupus erythematosus [5]. In view of these findings, interest in AGAG has been extended to other conditions not involving the connective tissue, and increased amounts have been found in patients with jaundice [6], neoplastic disease [7], diabetes mellitus [8], liver damage [9] and disseminated malignant diseases, particularly leukaemia [7].

Although the main pathological lesions of bilharziasis are found in the urogenital and digestive organs, any system or organ may be involved including the skin [10], cardio-pulmonary [11] and nervous system [12], the eyes [13] and the endocrine system [14].

These findings have prompted us to study the effect of bilharzial infection on connective tissue metabolism as reflected by the level and types of AGAG excreted in urine.

Materials and methods

Twenty-four-hour samples of urine were collected from adult male bilharziasis patients at different stages of the disease. Samples were collected from 19 patients with active urinary bilharziasis, 19 with clinical hepatosplenomegaly, 8 with clinical hepatosplenomegaly plus ascites, and 8 with hepatosplenomegaly plus ascites and oedema, all inpatients of Kasr El-Aini Hospital, College of Medicine, Cairo University. Twenty-four-hour samples of urine from fifteen normal adult males were used as control. These subjects had had no bilharziasis.

(1) Isolation of acidic glycosaminoglycans from urine

The method used for the isolation of acidic glycosaminoglycans was a modification of that of DIFERRANTE and RICH [15] and THOMPSON and CASTOR [6].

After 5% acetylpyridinium bromide had been added at a rate of 8 ml per 100 ml urine in the presence of 0.05 M sodium sulphate, the solutions were kept at 4°C overnight. The precipitates were collected by centrifugation and washed several times with 5 ml portions of 95% ethanol and extracted with 5 ml of 2 M sodium chloride and 1 ml of methanol by agitation. Insoluble material was centrifuged and extracted in the same way with 2 M sodium chloride. The supernatants were combined. The acidic glycosaminoglycans were then precipitated with 4 volumes of 95% ethanol at 4°C overnight.

The resulting precipitate was centrifuged and dissolved in 5 ml of distilled water and the hexosamine and uronic acid content was estimated according to DISCHE and BORENFREUND [16] and DISCHE [17], respectively.

Table I

Composition of samples of acid glycosaminoglycans isolated from 24-h-urine sample of normal subjects and bilharziasis patients at different stages of the disease

	No. of specimen	Chemical analysis, mg/24 h	
		Uronic acid	Hexosamine
Control	15		
mean		2.22	1.90
range		(0.98—3.46)	(0.93—2.88)
Stage I+	19		
mean		6.74	5.94
range		(2.44—11.04)	(1.84—10.04)
P		0.001	0.001
Stage II++	19		
mean		5.88	5.10
range		(2.96—8.50)	(2.85—7.35)
P		0.001	0.001
Stage III*	8		
mean		6.52	5.63
range		(2.34—10.70)	(3.08—8.18)
P		0.005	0.005
Stage IV**	8		
mean		1.14	1.19
range		(0.82—1.46)	(0.66—1.72)
P		0.005	0.005

+ Active urinary bilharziasis.
++ Hepatosplenomegaly.
** Hepatosplenomegaly plus ascites and oedema.
P Level of significance.

(2) Fractionation and characterization of acidic glycosaminoglycans isolated from urine

The isolated AGAG were incubated with trypsin and papain as described previously [15]. The digested protein was removed by SEVAG's technique [18], and the sample was dialyzed against distilled water at 4°C for 24 hrs, then the AGAG was reprecipitated by the addition of 4 volumes of 95% ethanol. The isolated purified AGAG was then subjected to fractionation as follows.

(3) Column chromatography on Dowex-1 X (200 400 mesh) in the chloride form

The resin was pretreated using the method of SCHILLER et al. [19], then a sample of purified AGAG dissolved in 5 ml distilled water was added. The column was then washed with distilled water (40 ml) and the sample was eluted stepwise with increasing concentrations of NaCl: 0.5 M, 1.25 M, 1.5 M, 2 M and finally 3 M (40 ml each). Fractions of 5 ml were collected. The eluates were assayed for AGAG contents by applying the carbazole method of DISCHE [17] for uronic acid and the anthrone method of ANDREANI and GRRAY [20] for hexoses.

(4) Paper chromatography of acid hydrolyzate of acidic glycosaminoglycans

For further characterization of the AGAG eluted from the Dowex-1 column, the eluate of each fraction was subjected to dialysis, then concentrated in vacuo at 40°C and hydrolyzed with 6N HCl in a sealed tube at 100°C for 4 hrs. The hydrolyzates were freed of HCl by distillation under vacuum at 40°C to dryness. The solid matter left in the flask was taken up in 0.1 ml of distilled water and a few drops of it were dropped on Whatman's No. 1 paper. The chromatograms were run in a single descending direction for 21 hrs in solvent I, butanol : pyridine : water (5 : 3 : 2 v/v) and solvent II ethylacetate : pyridine : water (12 : 5 : 4 v/v). Standard solutions of L-fucose, D-mannose, D-glucose, D-galactose, D-glucosamine and D-glucuronic acid were used as controls. Acid hydrolyzate of chondroitin sulphate was also used as galactosamine reference.

For detection of the sugars, air-dried chromatograms were treated with silver-nitrate reagent.

Results

The mean level of AGAG excreted in 14 hrs by both normal and bilharziasis patients at different stages of the disease are shown in Table I. Statistical analysis and the *t*-test indicated the increase in AGAG and were highly significant in all bilharziasis patients with the exception of those with hepatosplenomegaly plus ascites and oedema.

The type of AGAG excreted in normal and bilharzial urine was determined after fractionation of the purified AGAG on Dowex-1. When an aliquot of AGAG was applied to the column, three uronic acid positive fractions were eluted at 1.25 M, 1.5 M and 2 M NaCl fractions, respectively; a hexose-positive fraction was also eluted at 3 M NaCl.

The three uronic-acid-positive fractions were identified after dialysis, concentration, acid hydrolysis and chromatography, using the solvent systems I and II. The chromatograms revealed the presence of glucosamine in the eluates of the 1.25 M NaCl fraction, indicating the possible presence of heparitin sulphate (the amount of heparitin sulphate was determined in the eluted material by the method of DISCHE and BORENFREUND [16] as modified by LAGUNOF and WARREN [21], depending upon the determination of N-sulphate),

and galactosamine in the 1.5 M and 2 M NaCl fractions, indicating the possible presence of chondroitin sulphate.

The hexose-positive fraction eluted at 3 M NaCl was found to resemble keratan sulphate as indicated by the ratio of hexose to hexosamine (1 : 1).

The results of these analyses are summarized in Table II.

Table II

Types of acidic glycosaminoglycans in urine

Glycosaminoglycans	Normal percent of total	Bilharziasis percent of total
N-sulfated glycosaminoglycans (calculated as heparitin sulphate)	16.88	24
Keratan sulphate	5.13	3.22
Chondroitin sulphate	64.63	58.78

The fractionation results revealed that the major AGAG excreted by normal humans is chondroitin sulphate, with small amounts of heparitin sulphate and keratan sulphate as indicated in Table II.

AGAG excretion is considerably increased in bilharziasis. According to THOMPSON and CASTOR [6], this might be both a qualitative and a quantitative measure of the altered connective tissue metabolism.

We found that in bilharziasis heparitin sulphate is proportionately increased, while the amount of chondroitin sulphate and keratan sulphate are proportionately decreased.

Acknowledgements

The authors wish to thank Dr. EL-SAID H. EL-RASIKY, Department of Tropical Medicine, Faculty of Medicine, Cairo University, for permission to study patients under his care.

REFERENCES

1. BADIR, G.: Brit. J. Ophth. **30,** 215 (1946).
2. BERENSON, G. S., DALFERES, E. R.: Biochim. biophys. Acta (Amst.) **101,** 183 (1965).
3. DIFERRANTE, N.: J. clin. Invest. **36,** 1516 (1957).
4. DIFERRANTE, N., ROBBINS, W. C., RICH, C.: J. Lab. clin. Med. **50,** 897 (1957).
5. EL-GAREM, A. A.: Proc. 1st Int. Symp. Bilharziasis Part II, 539 (1962).
6. GHALIOUNGUI, P., SHAWARBY, K.: Proc. 1st Int. Symp. Bilharziasis Part II, 251 (1962).
7. GRADDOCK, J. G. Jr., KERBY, G. P.: J. Lab. clin. Med. **46,** 193 (1955).
8. HUNTER, G. W.: J. Parasit. **46** (2), 231 (1960).
9. KAWATA, N., KOIZUMI, T., WADA, R., YOSHIDA, T.: Gastroenterology **40,** 507 (1961).
10. LINKER, A., TERRY, K. D.: Proc. Soc. exp. Biol. (N. Y.) **113,** 743 (1963).
11. PEDRINI, V., LENNZI, L., ZAMBOTII, V.: Proc. Soc. exp. Biol. (N. Y.) **110,** 847 (1962).
12. RAGAP, M., HASHEM, M.: Proc. 1st Int. Symp. Bilharziasis Part II, 423 (1962).
13. RICH, C., MYERS, W. P. L.: J. Lab. clin. Med. **54,** 223 (1959).
14. THOMPSON, G. R., CASTOR, C. W.: J. Lab. clin. Med. **68,** 617 (1966).

15. DIFERRANTE, N., RICH, C.: Clin. chim. Acta **1,** 519 (1956).
16. DISCHE, Z., BORENFREUND, E.: J. biol. Chem. **184,** 517 (1950).
17. DISCHE, Z.: J. biol. Chem. **167,** 189 (1947).
18. SEVAG, M. G.: Biochem. Z. **273,** 419 (1934).
19. SCHILLER, S., SLOVER, G. A., DORFMAN, A.: J. biol. Chem. **236,** 983 (1961).
20. ANDREANI, D. V., GRRAY, C. H.: Clin. chim. Acta **1,** 7 (1956).
21. LAGUNOF, D., WARREN, G.: Arch. Biochem. **99,** 396 (1962).
22. WINAND, R. J.: J. clin. Invest. **47,** 2563 (1968).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (1—2), pp. 31—45 (1974)

PETIT MAL ABSENCES IN NIGHT SLEEP WITH SPECIAL REFERENCE TO TRANSITIONAL SLEEP AND REM PERIODS*

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(Received September 3, 1973)

Twelve all-night polygraphic records of 11 patients with PM epilepsy were analyzed. PM seizures appeared in the transitional periods from wake state towards sleep, during transient awakening and in the transitional periods to and from rapid sleep. Detailed analysis of the transitional periods by examination of the dynamics of four levels of vigilance in every 20 seconds, showed that PM absences occur after a fluctuation of the vigilance level and there is an optimum band of slightly depressed vigilance passing through which, especially in the direction from a more superficial to a deeper state, facilitates the occurrence of PM absences.

Numerous studies have been carried out concerning the relation of paroxysms with spike-wave discharges and sleep (GIBBS and GIBBS [10], JANZ [15], BATINI [2], NIEDERMAYER [20], ANGELERI et al. [1], PATRY et al. [24]). It is well-known since the work of GIBBS et al. [9] that slow sleep (SS) augments the number of spike-wave discharges. Modern polygraphic investigations have confirmed this statement. All investigators are unanimous in that falling asleep and superficial slow sleep stages (stage 1 and the beginning part of stage 2) facilitate the appearence of spike-wave paroxysms (SWp), while in deeper slow sleep the discharges undergo successive modifications. Although in these periods a great number of electric discharges is present, they do not appear in the type of SWp-s which are correlated with petit-mal paroxysms of the awake state (DELANGE et al. [5], CADILHAC et al. [4[, GASTAUT [7.], ROSS et al. [28], PASSOUANT and CADILHAC [22], STEVENS et al. [29], HALÁSZ and DÉVÉNYI [13]). During rapid sleep (RS) the early authors observed no or only a few discharges. Later, ROSS et al. [28] and PASSOUANT [23] found the same distribution but they emphasized the great similarity of the SWp-s in RS with the paroxysms of the awake state. PASSOUANT has pointed to the high probability of appearance in periods of transition between slow and rapid sleep. As regards RS periods, the poorly organized ones showed more frequent SWp-s. To gain a deeper understanding of the correlation of SWp-s and the level of vigilance, it seemed

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necessary to distinguish in sleep records between SWp-s which are correlated with petit-mal paroxysms and electric discharges; and to analyze in greater detail the correlation of petit-mal absences and RS periods. Present paper reports on such a study.

Material and methods

The data presented were yielded by 12 all-night polygraphic examination of 11 patients. All had typical petit-mal absences as the leading manifestation of their illness and from the EEG point of view, synchronous bilateral SWp-s. The clinical data of the patients are summarized in Table I. Nine patients had frequent attacks and were in a poor condition; one patient had few, and one had no seizures during the examinations. Anticonvulsive treatment was suspended 24—48 hours before the examination. Most patients were spending their first night in the laboratory, and only some were well-accustomed to laboratory circumstances.

For the polygraphic examinations, a 16-channel ALVAR EEG apparatus was used. The EEG, EOG (1 or 2 leads) the EMG (chin and/or masseter), and in some cases the EDG, were recorded from skin electrodes. During the registration, behaviour and motor activity of the patients were followed by television inside the sleep-boxes by an ultrared camera. Three patients were stimulated by auditory stimuli (hand claps) given at random in every phase of sleep and in two further sleep records the EEG reactions to arousal provoked by spontaneous body movements, were also analyzed.

In 10 records of 9 patients the transitional periods between slow and rapid sleep and the rapid-sleep periods were analyzed together in more detail. In these periods every 20 seconds were examined and the following factors were registered: the presence of external or internal arousal influence (auditory stimuli and body movements), sleep-spindles and/or K-complexes, muscular tone, eye movements, alpha rhythms.

According to the presence or absence and the combinations of these factors, four levels were distinguished in every period consisting of transitional sleep-rapid sleep-transitional sleep (TrS-RS-TrS). The levels were 1. REM in the presence of rapid eye movements and absence of muscular tone with rapid activity in the EEG; 2. RS in the same situation without eye movements; 3. transitional sleep$_1$ (TrS$_1$) in the presence of "micro-awakening" reactions consisting of alpha-rhythms, with or without muscular tone; and 4. transitional sleep$_2$ (TrS$_2$) in the presence of sleep-spindles and/or K-complexes, with or without muscular tone. The distribution of the four levels in 50 periods of TrS-RS-TrS was estimated and the dynamics of the alternations between the levels were analyzed. For comparison, 5 nights of 3 healthy control subjects were analyzed in the same manner (23 TrS-RS-TrS periods).

In all estimations, only the SWp-s similar to those in the wake state were considered, since spike-wave discharges with transformed morphology have been reported previously (HALÁSZ and DÉVÉNYI [13]).

Results

Regarding the distribution of SWp-s in the course of sleep, the following periods proved to have a promoting effect (Table II and Fig. 1 a, b): a) falling asleep; b) light slow sleep (the beginning of stage two); c) transitional periods from slow towards rapid sleep and from rapid towards slow sleep, i.e. previous to and following the RS periods; d) transitory wake states during night.

In the majority of the patients, the frequency of SWp-s was higher in the second than in the first half of the night. In 9 patients SWp-s appeared also in the RS periods while in two, their number in RS was even higher than in TrS or SS periods. (These data will be dealt with in detail.) The patients with more frequent absences during the day and a worse clinical course had more SWp-s in TrS-RS-TrS periods than those with less frequent seizures (Fig. 7).

The organization of sleep was particular from two points of view. Transitory wake periods were unusually frequent; it seemed that they were prolonged by the seizures occurring during their course. This is a vicious circle:

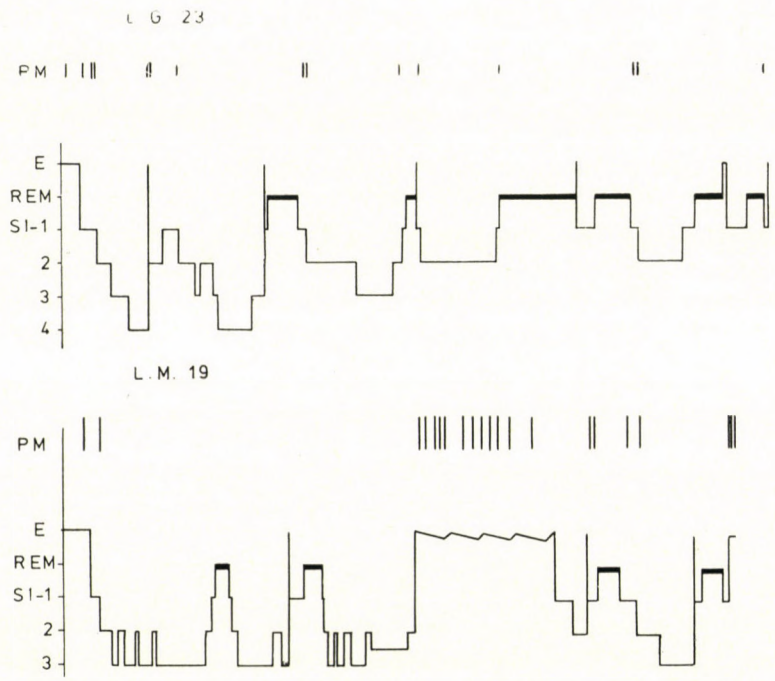


Fig. 1a, b. Typical hypnograms of PM patients (vertical lines represent PM seizures)

after awakening in drowsiness, SWp-s appear and often they have an awakening effect, again followed by drowsiness with a new attack, and so on (Fig. 1b).

Another striking feature is the high percentage of TrS before and after RS periods (Table II). This finding was less evident in patients who met the criteria of a comparatively good clinical condition.

Distribution of SWp-s during nocturnal sleep and the above-mentioned features of the sleep organization of petit-mal patients show that:

a) the appearence of SWp-s is facilitated in the transitional periods between wakeness and slow sleep and slow sleep and rapid sleep;

b) there must exist a dynamic process which has a trigger effect on SWp-s.

To gain a better understanding of these processes, the transitional periods between slow and rapid sleep and the RS periods were examined in detail. The TrS-RS-TrS periods of 9 patients' 10 nights were analyzed every 20 seconds and according to the EEG and polygraphic signs enumerated in the methodological part, four levels of vigilance were distinguished. REM level

Table I

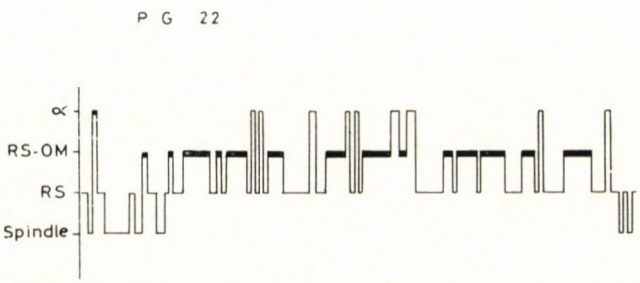
Name	Sex	Age, years	Beginning of disease, years	Duration of illness, years	Seizures				Treatment
					PM	GM	Tonic-atonic	Frequency	
V—V.M.	♀	24	12	12	+	+ (rare)	—	2—3/week	ethosuximide phenobarbital
J.M—H.	♀	22	4	18	+	+ (rare)	—	without	phenobarbital amphetamine
F. P.	♂	11	10	1	+	+	—	1/day	diphenylhydantoin phenobarbital
L. G.	♀	22	13	9	+	+	—	14/day	sodium di-propyl acetate
G.M—A.	♀	12	7.5	4.5	+	+	+	20/day	diphenylhydantoin phenobarbital
B. P.	♂	16	12	4	+	+	+	several/day	ethosuximide carbamazepine phenobarbital
P. J.	♀	23	4	19	+	+	+	30/day	phenobarbital diazepam
V. F.	♂	6	3	3	+	—	—	15—20/day	ethosuximide phenobarbital
M. E.	♀	29	6	23	+	+	+	several/day	diazepam phenobarbital amphetamine
L.M—C.	♀	19	13	6	+	+	+	1—5/day	diphenylhydantoin sodium di-propyl acetate
T. J.	♀	15	9.5	5.5	+	+	+	1/month	diphenylhydantoin phenobarbital

corresponds to rapid sleep with rapid eye movements; RS level to rapid sleep without eye movements, while TrS_1 to the transitional period from rapid sleep towards wake state, and TrS_2 to the transition from rapid sleep towards slow sleep. Hence we gain a dynamic picture of the distribution of the levels and fluctuations in the dimension of vigilance (Fig. 2a, b, c).

The appearence of SWp-s was most pronounced in the TrS_2. A TrS_2 following the REM period was richer in SWp-s than the preceding period (5 and 12%, respectively). The number of SWp-s in TrS_1 was less than half of those in TrS_2. Nearly the same was true for the RS level, while in REM, SWp-s occurred rarely (Fig. 3).

The distribution of the four levels in the whole TrS-RS-TrS period was different from that in control subjects. The TrS levels, and especially the TrS_2

Mental state		EEG					Course			Serial number of night
					Focal signs					
good	bad	spike-wave 3 c/s	spike-wave slow	rapid discharge	yes	no	progressive	stationary	regressive	
+	−	+	−	−	−	+	−	+	−	1.
+	−	+	−	−	−	+	−	−	+	1.
+	−	+	−	−	+ (clinically)	+ (EEG)	−	+	−	1.
+	−	+	−	−	−	+	+	−	−	2.
−	+	+	−	−	+ (clinically) + EEG	−	+	−	−	3.
−	+	+	+	−	+ pituitary tumour	−	+	−	−	1.
+	−	+	−	−			−	+	−	2.
−	+	+	−	−	+ (clinically)	+ (EEG)	+	−	−	1.
−	+	+	−	+	−	+	+	−	−	7.
−	+	+	−	+	+ (EEG)	−	+	−	−	1.—4.
+	−	+	−	−	−	+	−	−	+	3.



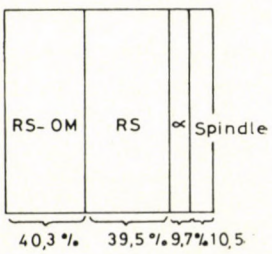


Fig. 2a

Table II

Patients	Slow sleep				Transitional sleep		Rapid sleep	
	1		2					
	number of PM-s	duration, minutes	number of PM-s	duration minutes	number of PM-s	duration minutes	number of PM-s	duration minutes
J. M—H	1	6	—	280	3	39	—	127
V—V.—M	—	12	—	121	—	28	—	44
G. M.	1	20	—	101	12	76	5	118
T. J.	3	36	—	240	2	57	1	68
M. E.	2	31	—	133	4*	64	1	90
V. F.	2	8	1	136	12	61	21	116
B. Ph.	1	48	—	142	11	101	5	128
F. P.	1	3	10	162	3	30	1	145
P. J.	4	37	—	50	7	54	24	191
L. G.	3	15	2	182	6	87	1	110
L.M—C.	3	41	2	122	5	59	2	74
L.M—C.	—	8	1	89	8	98	—	84

* tonic seizures.

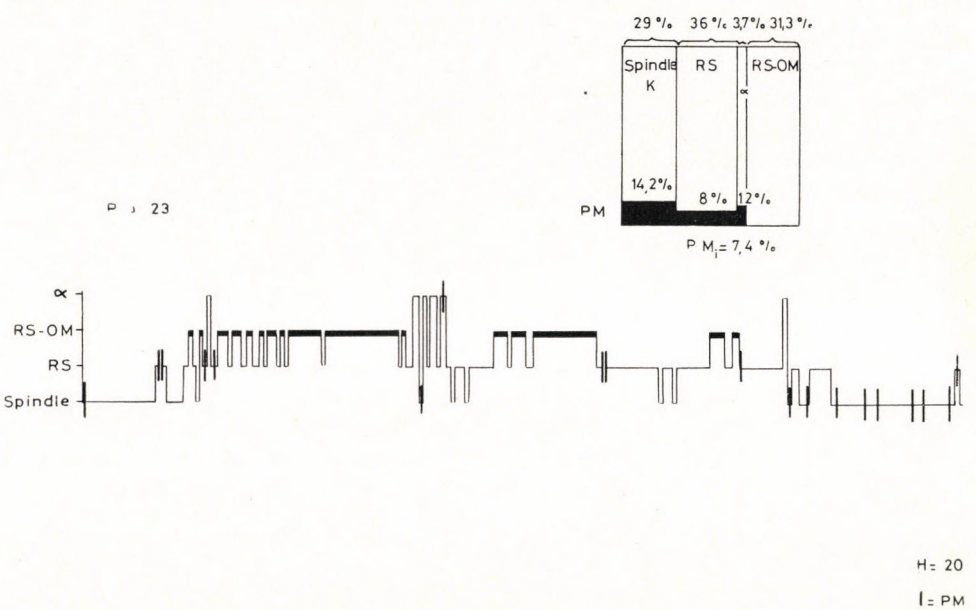


Fig. 2b

Awake at night		Awakening period after night sleep		Total					
				Night sleep		first part		second part	
number of PM-s	duration minutes	number of PM-s	duration minutes	number of PM-s	duration minutes	number of PM-s	duration minutes	number of PM-s	duration minutes
—	3	—	1	4	499	2	129	2	130
23	186	5	9	28	533	2	266	26	267
—	—	—	1	18	533	1	266	17	267
1	38	—	1	7	602	6	301	1	301
1	91	—	1	8	460	3	230	5	230
9	76	2	2	45	519	19	259	26	260
3	83	—	—	19	588	—	294	19	294
1	8	—	13	16	545	3	272	13	273
—	—	7	37	42	462	14	231	28	231
2	6	—	1	13	542	8	271	5	271
14	124	—	—	26	605	2	302	24	303
—	—	—	8	9	473	1	236	8	237

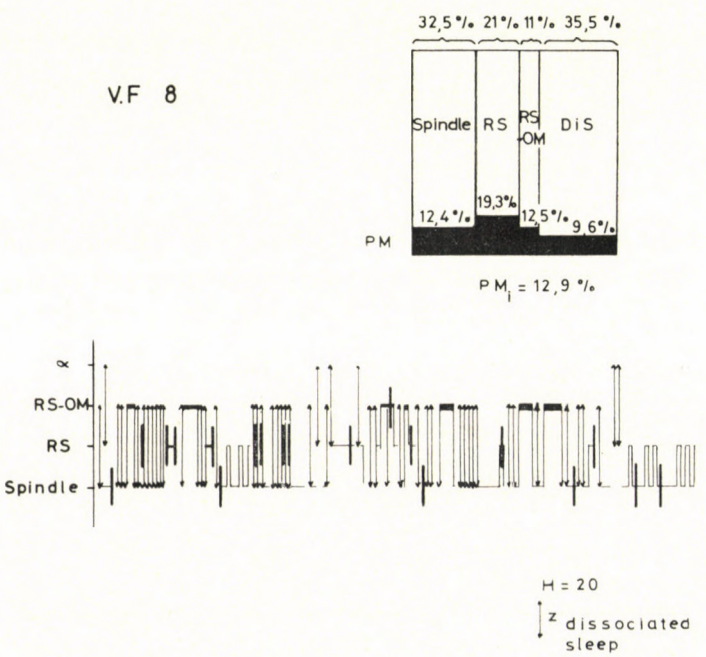


Fig. 2c. Typical TrS-RS-TrS periods in control subject (a), and in PM patients (b and c)

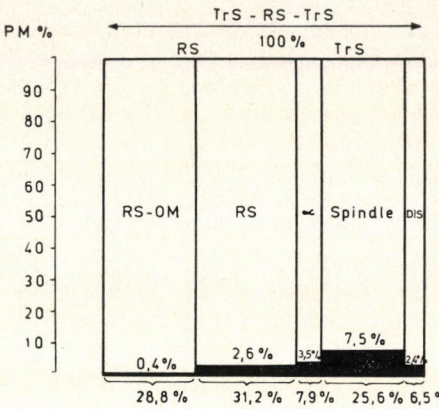


Fig. 3. The amount of PM paroxysms at different levels of TrS-RS-TrS periods distributed among 12 nights of 11 patients

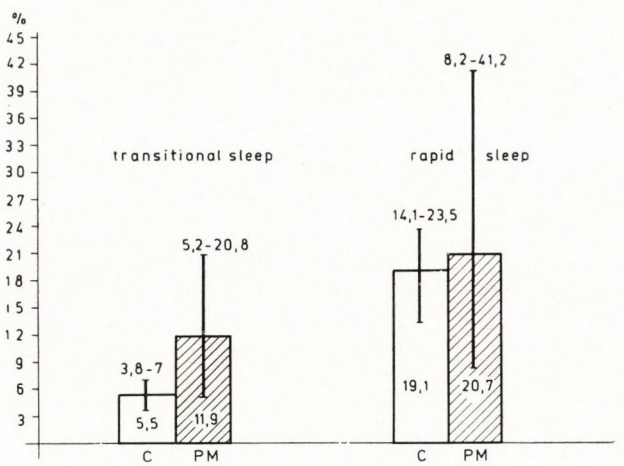


Fig. 4. Duration of transitional sleep and rapid sleep during 12 nights of 11 patients and during 5 nights of 3 control subjects

level had conspicuously more in petit-mal patients than in control subjects (Figs 4 and 5).

The TrS-RS-TrS period was characterized by great fluctuations, especially towards slow sleep even on TrS and REM levels (Fig. 6).

The REM was again and again interrupted by brief transitions to TrS. Much "dissociated sleep" occurred: eye movements appeared together with sleep spindles and/or K-complexes or with muscular tone (Fig. 1c).

In the rare cases when SWp-s appeared in REM (4 REM-s of 4 patients), the whole TrS-RS-TrS period was richer in SWp-s than the other TrS-RS-TrS periods of the same patient. They showed the greatest number of SWp-s among

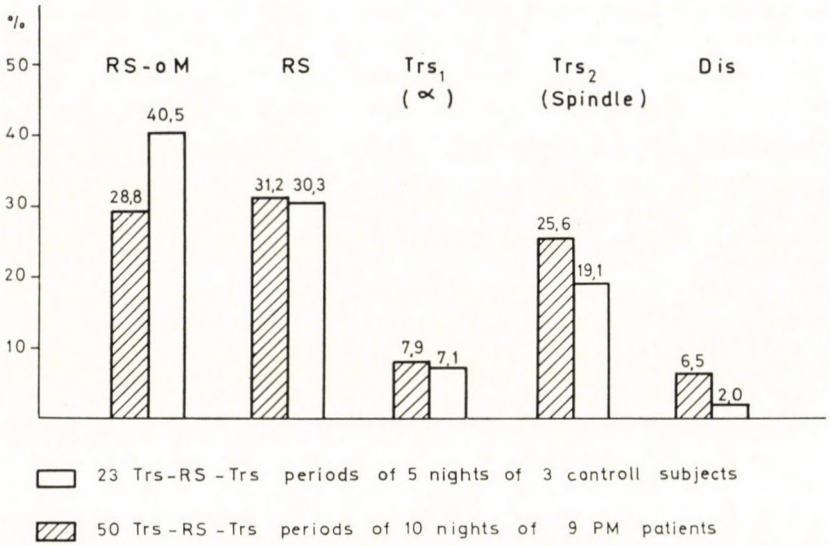


Fig. 5. Distribution of the different levels within TrS-RS-TrS periods of PM patients and of control subjects

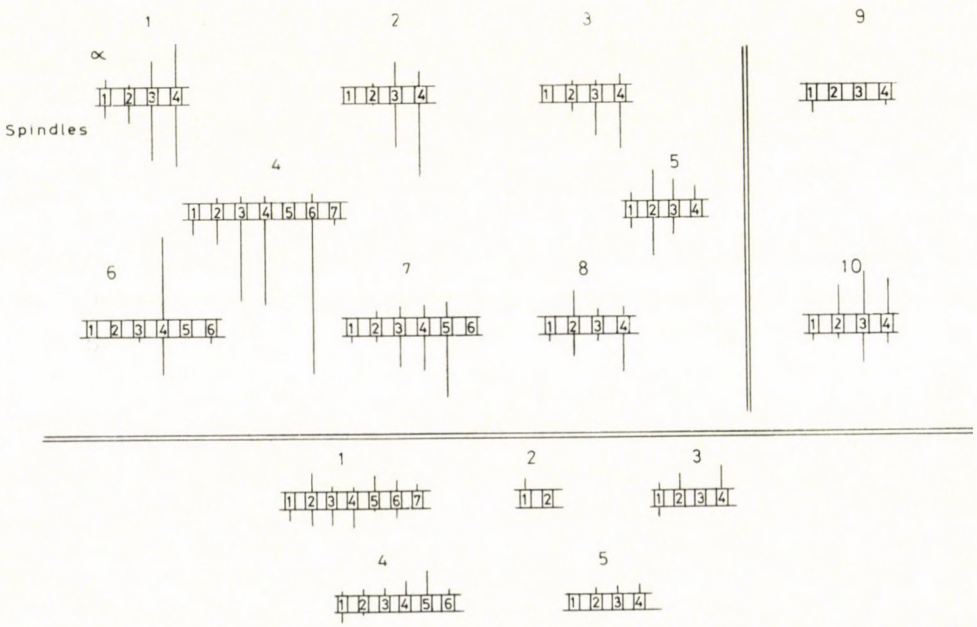


Fig. 6. Number of fluctuations towards wake state (upwards) and slow sleep (downwards) in TrS-RS-TrS periods during nocturnal sleep of PM patients (upper part) and of control subjects (lower part)

all the TrS-RS-TrS periods of all patients (Fig. 7). SW-s never appeared during the prolonged runs of eye movements, except in connection with random groups of eye movements usually at the beginning and at the end of RS periods. During SWp-s, rapid eye movements seemed to be inhibited and the most common sequence of events was that SWp-s appeared after a silent period which was preceded by a group of eye movements.

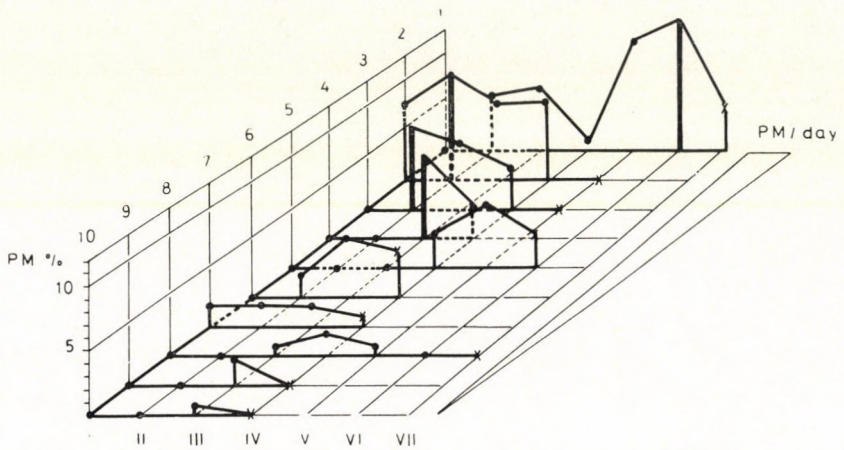


Fig. 7. Distribution of PM paroxysms in TrS-RS-TrS periods during nocturnal sleep of 10 patients arranged in order of PM/day. The perpendicular plans represent 10 nights of 9 patients and the horizontal plan the sequence of TrS-RS-TrS periods. In every period, PM% is plotted. The triangle on the right represents the amount of absences/day

The first and the last TrS-RS-TrS periods of the night were different. The first was frequently lacking SWp-s and the last contained more fluctuations towards the wake state than the others.

Three patients were stimulated by randomly given acoustic stimuli in every phases of their sleep. One patient was stimulated more than fifty, two patients more than a hundred, times. The stimuli evoked spike-wave answers only in slow sleep, stage 2 at TrS and at some RS levels. The spike-wave answers were not paroxysms but spike-wave groups containing more than two spike-wave complexes. As regards slow sleep stage 2, they were more frequent in its superficial parts. The records of five patients (including the previous 3) were analyzed for the arousal reactions provoked by spontaneous body movements during the night. In two records, more than sixty, and in one, more than hundred movements were counted. Body movements as well as acoustic stimuli evoked spike-wave answers at the same sleep stages. The percentage of spike-wave answers to arousal influence is plotted against the sleep stages in Fig. 8. By means of acoustic stimuli applied during a SWp, this was inhibited, though a preceding stimulus evoked a spike-wave answer in the same sleep

phase. The spike-wave answer seemed to appear after an oscillation of the level of vigilance. On the reactive level of vigilance (from TrS_1 to slow sleep, stage 2) the stimulus depending on the starting level induced either a transient desynchronization followed by a sleep spindle and/or a K-complex answer, or immediately evoked a sleep spindle or/and a K-complex reaction which was again followed by relatively desynchronized spindling. The oscillations appeared to be prolonged and slow with several overswings and rebounds especially in answers provoked by body movements. Spike-wave answers appeared either after transient desynchronization or after a sleep-spindle and/or a K-complex answer, but always at a distinct level of vigilance. They occurred when the patient passed through this level with a decline of a certain slope, starting either from a deeper or a more superficial level of vigilance.

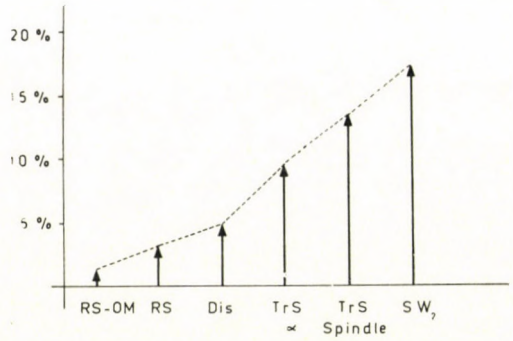


Fig. 8. Amount of spike-wave reactions to acoustic stimuli and body movements derived from the arousal effect evoked by 299 acoustic stimuli and 260 body movements (3 and 5 patients, respectively)

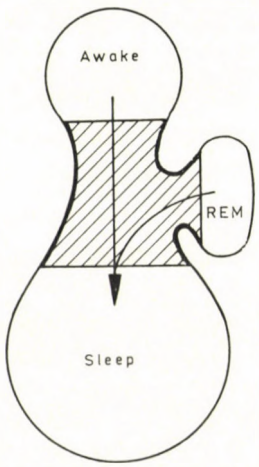


Fig. 9. Schematic view of the site of PM facilitation in the sleep-wakefulness continuum (striped area). The arrows represent the direction of changes in the level of vigilance during which PM absences mainly occur

Discussion

The first conclusion is that in nocturnal sleep SWp-s occur in the transitory periods from wakefulness towards slow, and from slow towards rapid sleep (Fig. 9). This may happen in the course of the spontaneous fluctuations of vigilance or be evoked by arousal influences provoking a partial wakening followed by sinking into sleep again. For the latter the most superficial levels of slow sleep proved to be especially apt.

These phenomena indicate that a certain optimum level of vigilance must exist at which SWp-s appear. Furthermore, especially the passage through this level is what seems to be a promoting factor of SWp-s. This particular level of vigilance is neither a wake state nor sleep. It probably begins with a quiet wakefulness and extends through drowsiness and transitional sleep periods into the realm of superficial slow sleep. We are inclined to believe that without a previous oscillation of the level of vigilance, SWp-s never appear, or, in other words, a fluctuation of the level of vigilance must precede the bursts of SWp-s. There is an oscillation of vigilance between the levels bordering the optimum period, where SWp-s mostly appear. During this process oscillations directing towards slow sleep proved to be more facilitatory for SWp-s than the oscillations towards awakening. Turning the balance towards RS had the least provoking effect among the fluctuations. These statements are supported by the following facts.

1. Falling asleep has a strong facilitatory effect for SWp-s;

2. at TrS_2 levels following RS levels – in the transitional state from RS towards slow sleep — there were more SWp-s than in the preceding TrS_2 periods;

3. TrS_2, the fluctuation towards slow sleep, is more facilitatory for SWp-s than the TrS_1 levels which reflect the fluctuations towards awakening;

4. arousal stimuli evoke spike-wave answers in the phases characterized by a rebound towards slow sleep after a transient awakening.

The other characteristic feature of the nocturnal sleep of petit-mal patients, the persisting wake states and the high percentage of TrS, are closely connected with the SWp-s. The transitional balance allows SWp-s to occur and the paroxysms themselves prolong the state of balance and a vicious circle develops.

It must be assumed that in certain pathological circumstances the transitional periods between the three states of existence, namely the wake state, slow sleep and rapid sleep, are augmented. LAIRY et al. [17] found an augmentation of transitional sleep in psychopathological processes. Evaluation of sleep records containing augmented transitional periods on the basis of the criteria for dividing sleep into rapid and slow periods, as proposed by the manual of RECHTSCHAFFEN and KALES [27], is no more sufficient. Therefore,

in order to find a quantitative and at the same time dynamic method for analyzing transitional periods and the fluctuations in RS, we have elaborated our factorial method and classification of the level of vigilance in transitional periods of PM patients. The amount of TrS in healthy subjects estimated by our method corresponds to the data of SNYDER (quoted by LAIRY et al. [17]). The parallelism in augmented TrS between certain psychopathological processes, especially those with troubles of consciousness and PM patients, is striking and there may be some common disturbance in the function of the reticular arousal systems. However, the role of the different psychotropic drugs, and in the PM group, even the role of the seizures themselves producing these phenomena, cannot be neglected.

Our data agree well with the above-mentioned findings in that a certain lowering of the level of vigilance facilitates the occurrence of SWp-s. In experimental situations, a lowering of the level of vigilance was also a necessary factor in eliciting synchronous bilateral spike-wave discharges (POLLEN et al., [25]). On the other hand, arousal effects and, in animal experiments, excitation of the mesencephalic reticular arousal system, proved to exert a potent inhibitory influence on SWp-s (JUNG [16]; GUERRERO et al. [11]; POLLEN et al. [25]). The data connected with the triggering effect of awakening (LEHMANN [18], NIDERMAYER [20], JANZ [15]) can be interpreted in the same way as our results described above.

It seems that the interpretation derived from sleep studies may well explain the occurrence and distribution of SWp-s during the day. There are data to demonstrate the enhancing effect of inattention, boredom and drowsiness on petit-mal absences (MIRSKY and VAN BUREN [19]; BUREAU et al. [3]; GUEY et al. [12]). It is likely that slight fluctuations in the level of vigilance during the day contribute to the appearance of SWp-s. The well-known triggering effect of emotional events upon petit-mal absences is probably connected with a rebound effect following an intensive arousal influence produced by stressor stimuli.

The correlation between REM and SWp-s requires particular attention. Generally speaking, we found an antagonism between rapid eye movement periods and SWp-s. In some cases, however, groups of eye movements seemed to be somehow in connection with SWp-s. These constellations were always observed at the beginning or the end of REM periods. If we consider the REM to represent a level of vigilance near to the wake state, as supported by different data (OKUMA and FUJUMARI [21]; EVARTS [6]; POMPEIANO [26]), or even as a state during which the cortex is working in a different way but at a level similar as in wakefulness, we obtain an explanation of the antagonism with SWp-s. The thalamic reticular system is also inhibited during full wakeness and during REM, as shown by the depression of thalamic recruitment (GUAGUINTO [8]). At the beginning or at the end of REM this high level of vigil-

ance is not yet developed or not any more maintained and a transient arousal effect provoked by the emotional stress of a dream corresponding to a short eye-movement group, can be followed by a rebound fluctuation with the possible appearance of SWp-s.

According to our findings and to data in the literature, the correlation of SWp-s with the level of vigilance must be considered to represent a crucial point in the mechanism of petit-mal absences. This indicates that the pathophysiological processes behind petit-mal absences are closely connected with the sleep–vigilance system. In each epileptic process two main factors can be distinguished, viz. 1. a local epileptic dysfunction of certain cell groups, or certain cerebral systems caused by a damage of external origin or by a functional disturbance on the neurochemical level; 2. a general, probably genetically determined tendency to convulsions. In petit-mal epilepsy, the focal disturbance is usually hidden. However, in many cases, focal electrical and/or clinical signs have been found. The sites of these focal processes located by means of stereoencephalography, as well as the regions from which secondary bilateral spike-wave synchronisation is often triggered and the areas from where electrical or chemical stimulation induces generalized spike-wave paroxysms in animal experiments, are all connected with fields having a triggering driving effect upon the sleep system (for references see HALÁSZ, [14]). It may be assumed that the genetically determined factor in petit-mal epilepsy consists in some functional disturbance of the reticular arousal system. Our finding that in petit mal patients transitional sleep is prolonged and the conspicuously great fluctuations of the level of vigilance towards slow sleep on REM levels as compared with those in healthy control subjects, is interpreted as a reflection of the dysfunction which increases the possibility of a triggering of the sleep system by an epileptic focus.

Acknowledgements

Authors wish to thank professor P. PASSOUANT for his continued support and advice during the course of this work.

REFERENCES

1. ANGELERI, F., BERGONZI, P., FERRONI, A.: A statistical study of the amount of diffuse synchronous spike and wave activity during nocturnal polygraphic recording in epileptics. Electroenceph. clin. Neurophysiol. 25:85—86, 1968.
2. BATINI, C.: Nocturnal sleep in patients presenting epilepsy with bisynchronous EEG discharges. Electroenceph. clin. Neurophysiol. 14:957—958, 1962.
3. BUREAU, M., GUAY, J., DRAVET, C., ROGER, J.: A study of the distribution of the petit mal absences in the child in relation to his activities. Electroenceph. clin. Neurophysiol. 25:513, 1968.
4. CADILHAC, J., VLAHOVITCH, B., DELANGE, M.: Considerations on the changes in epileptic discharges during the phase of eye movements. Electroenceph. clin. Neurophysiol. 18:96, 1965.
5. DELANGE, M., CASTAN, P., CADILHAC, J., PASSOUANT, P.: Étude du sommeil de nuit au cours d'épilepsies centrencéphaliques et temporales. Rev. Neurol. 106:106—113, 1962.
6. EVARTS, E. V.: Activity of individual cerebral neurons during sleep and arousal. Res. Publ. Ass. nerv. ment. Dis. 45:319, 1967.

7. GASTAUT, H.: An EEG study of nocturnal sleep in epileptic patients. Electroenceph. clin. Neurophysiol. 18:96, 1965.
8. GUAGUINTO, S.: Changes in the threshold of the recruiting responses during sleep and wakefulness: a quantitative study. Arch. ital. Biol. 106:364, 1968.
9. GIBBS, F. A., DAVIS, H., LENNOX, W. G.: The electroencephalogram in epilepsy and in conditions of impaired consciousness. Arch. Neurol. Psychiat. (Chic.) 34:1133—1148, 1935.
10. GIBBS, F. A., GIBBS, E. D.: Atlas of electroencephalography Vol. II. Addison-Wesley Press, Cambridge, Mass., 1952.
11. GUERRERO-FIGUREOA, R., BARROS, A., DE BALBIAN VESTER, F.: Some inhibitory effects of attentive factors on experimental epilepsy. Epilepsia (Aust.) 4:225—240, 1963.
12. GUEY, J., BUREAU, M., DRAVET, C., ROGER, J.: A study of the rhythm of petit mal absences in children in relation to prevailing situations. The use of EEG telemetry during psychological examinations, school exercises and periods of inactivity. Epilepsia (Amst.) 10:441—451, 1969.
13. HALÁSZ, P., DÉVÉNYI, É.: Az epilepsziás generalizált tüske-hullám synchronisatio viselkedése természetes éjszakai alvásban. Ideggyógy. Szle. 24:490—508, 1971.
14. HALÁSZ, P.: The epileptic generalized spike-wave mechanism and the sleep wakefulness system. Acta physiol. Acad. Sci. Hung. 42:293—314, 1972.
15. JANZ, D.: „Aufwach-Epilepsien" (als Ausdruck einer den „Nacht"-oder „Schlaf"-Epilepsien gegenüberzustellenden Verlaufform epileptischer Erkrankungen). Arch. Psychiat. Neurol., 191:73—85, 1953.
16. JUNG, R.: Correlations of bioelectrical and autonomic phenomena with alteration of consciousness and arousal in man. Symposium on "Brain mechanisms and consciousness" Blackwell, Oxford 1954. Pp. 310—344.
17. LAIRY, C. G., BARROS FEREIRA, M., GOLDSTEINAS, L.: Les phases intermédiaires du sommeil. Proc. XV. European Meeting on Electroencephalography, Bologna, 1967.
18. LEHMANN, H. J.: Präparoxismale Weckreaktion bei pyknoleptischen Absences. Arch. Psychiat. Nervenk. 204:417—426, 1963.
19. MIRSKY, A. F., VAN BUREN, J. M.: On the nature of the "absences" in centrencephalic epilepsy: a study of some behavioral electroencephalographic and automatic factors. Electroenceph. clin. Neurophysiol. 18:334—348, 1965.
20. NIEDERMAYER, E.: Sleep electroencephalograms in petit mal Arch. Neurol. (Chic.) 12:625—630, 1965.
21. OKUMA, T., FUJUMARI, M.: Electroencephalographic and evoked potential studies during sleep in the cat. I. The study on sleep. Folia psychiat. neurol. Jap. 17:25, 1963.
22. PASSOUANT, P., CADILHAC, J.: Décharges épileptiques et sommeil. Epilepsy, Mod. Probl. Pharmacopsychiat. 4:87—104 (Karger, Basel, New York) 1970.
23. PASSOUANT, P.: Physiopathologie du sommeil rapide. Psychol. méd. 4:439—448, 1972.
24. PATRY, G., LYAGOUBI, S., TASSINARI, A.: Subclinical "electrical status epilepticus" induced by sleep in children. Arch. Neurol. (Chic). 24:242—252, 1971.
25. POLLEN, D. A., PEROT, P. H., REID, K. H.: Experimental bilateral wave and spike from thalamic stimulation in relation to level of arousal. Electroenceph. clin. Neurophysiol. 15:1017—1028, 1963.
26. POMPEIANO, O.: Sleep mechanisms. In "Basic Mechanisms of the Epilepsies" Little Brown et Co., Boston 1969.
27. RECHTSCHAFFEN, A., KALES, A.: A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. National Institutes of Health Publication No 204, Bethesda, Md. 1968.
28. ROSS, J. J., JOHNSON, L. C., WALTER, R. D.: Spike and wave discharges during stages of sleep. Arch. Neurol (Chic.) 14:399—407, 1966.
29. STEVENS, R. J., KOMADA, H., LONSBURY, B., MILLS, L.: Ultradian characteristics of spontaneous seizures discharges recorded by radio telemetry in man. Electroenceph. clin. Neurophysiol. 31:313—325, 1971.

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (1—2), pp. 47—49 (1974)

QUANTITATIVE ESTIMATION OF LIPIDS IN NEEDLE BIOPSY SIZED SPECIMENS OF CADAVER LIVER

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(Received September 13, 1973)

Total lipids were determined gravimetrically in gram-sized samples of 107 cadaver livers. Cholesterol, triglycerides and phospholipids were estimated from extracts of 10—20-mg samples of the same livers. Total lipids determined in macro-samples and the sum of lipid fractions obtained in the micro-samples were in good agreement. Normal livers contained at most 5.5% fat per wet weight. Higher values were due to fatty degeneration. The fact that in fatty degeneration triglyceride accumulation is not accompanied by a significant increase in cholesterol or phospholipid content, has been confirmed.

The procedure permits estimation of lipid fractions in samples obtained *in vivo* by the Menghini biopsy needle.

The value and justification of liver needle biopsy in dubious cases in which the usual clinical and laboratory methods fail to clarify the diagnosis, is unquestionable (MAGYAR and FISCHER [13]; KALK [8]; LEEVY [12]; SZARVAS et al. [18]). Histological estimation of the degree of fatty degeneration is somewhat subjective (BERINGER et al. [1]; IRSIGLER and HRABAL [6]; KREMER [9]; JAROSS et al. [7]). Therefore, a method for the quantitative estimation of the main fractions of liver lipids suitable for the analysis of needle biopsy material has been devised.

Material and method

Total liver lipids were estimated gravimetrically in 107 unselected cadaver livers. About 1 g of liver tissue was homogenized and extracted according to FOLCH et al. [3]. After evaporation, the total amount of lipid was measured on an analytical balance. The results served as reference for judging the reliability of data obtained with tissue samples of mg size.

Gravimetric measurement of total lipids from tissue specimens of 10—20 mg, equal to needle biopsy samples, presents problems since the assay would require a balance of μg sensitivity.

In 76 microspecimens total lipids were estimated by the phospho-vanillin procedure (POSTMA and STROES [15]). However, insufficient sensitivity, and the disturbing effect of sphingosin (SAIFER and FELDMAN [17]) made this method unsuitable for tissue-lipid determination. Therefore, to obtain the total lipid content of biopsy-sized samples, chemical estimation of the main lipid classes was undertaken.

From 107 livers, subjected also to gravimetric estimation, samples weighing 4.2 to 46.5 mg were extracted according to FOLCH, and after evaporation of the solvent the residue was re-dissolved in 2 ml of hexane, and total cholesterol (RAPPOPORT and EICHHORN [16]), triglycerides (VAN HANDEL and ZILVERSMIT [4]), and phospholipids (YOUNGBURG and YOUNGBURG [19]) were determined. The results were expressed in percentage of tissue wet weight.

The livers after formalin fixation, paraffin-embedding, and haematoxylin-eosin staining (KREMER et al. [10]), were examined also histologically.

Results and discussion

The total of lipid fractions obtained in micro-samples was in good agreement with the results obtained by gravimetry of larger tissue samples. Chemical determination of the lipid fractions in tissue samples of less than 10 mg were, however, not reliable.

Table I

Author(s)	No. of cases	Age, year	Fat content, percent (gravimetric)	Total cholesterol	Triglycerides	Phospholipids	Sum of lipide fractions	Tissue weight, mg
				in per cent of tissue wet weight				
DONHOFFER	107	0—87	1.7—25.6	0.11—0.84	1.3—26.4	0.4—3.60	1.81—30.84	4.2—46.5
KREMER et al. [10]	150	—	— —	0.15—1.12	0.5—24.4	1.05—3.60	1.70—29.12	— —
KWITEROWICH et al.* [11]	12	4—61	— —	0.20—0.54	0.29—5.43	2.10—3.07	2.59—9.04	— —

* Examined only normal livers.

The results agreed with data in the literature (Table I). Total fat of the normal liver may amount to as much as 5.5% of wet weight. A fat content above 8.0% is a reliable sign of a pathological fatty liver. Confirmation was obtained that in fatty degeneration only the triglycerides are accumulated with any considerable increase in cholesterol or phospholipids (POGGI and DI LUZIO [14]; CARLSON et al. [2]; IRSIGLER and HRABAL [6]; KREMER [9]; KREMER et al. [10]). Therefore, if only the degree of fatty degeneration has to be established, estimation of esterified fatty acids — as proposed by HOEFLMAYR and FRIED [5] — should be the method of choice. Esterified fatty acids include fatty acids in esterified cholesterol and phospholipids. However, the amount of the latter does not change significantly in fatty degeneration, so the increase in esterified fatty acids represents the accumulation of triglycerides.

This statement does not, however, apply to storage diseases, in which different lipids are accumulated in the liver. Therefore, in these cases separate determination of the lipid groups is the correct procedure.

As has been shown, the lipid fractions can be determined by chemical methods in 10—20-mg samples of cadaver liver. Good results have also been obtained in Menghini needle-biopsy material taken *in vivo* (JOBST and DONHOFFER, in preparation).

LITERATURE

1. Beringer, A., Hrabal, I., Irsigler, K., Thaler, H.: Der Einfluß von Tolbutamid auf die diabetische Fettleber. Dtsch. med. Wschr., **92,** 2388—2392 (1967).
2. Carlson, L. A., Liljekahl, S. O., Wirsén, C.: Blood and tissue changes in the dog during and after excessive free fatty acid mobilization. Acta med. scand., **178,** 81—102. (1965).
3. Folch, J., Lees, M., Sloane Stanley, G. H.: A simple method for the isolation and purification of total lipids from animal tissues. J. biol. Chem., **226,** 497—509 (1957).
4. Handel, E. van, Zilversmit, D. B.: Micromethod for the direct determination of serum triglycerides. J. Lab. clin. Med., **50,** 152—157 (1957).
5. Hoeflmayr, J., Fried, R.: Quantitative Methode zur Erfassung des Verfettungsgrades der Leber durch Bestimmung der veresterten Fettsäuren. Klin. Wschr., **50,** 657—658 (1972).
6. Irsigler, K., Hrabal, I.: Zur Neutralfettbestimmung im Biopsiematerial der menschlichen Leber. Klin. Wschr., **46,** 432—438 (1968).
7. Jaross, W., Hanefeld, M., Stötzer, H.: Diagnostische und pathophysiologische Aussagen durch Mikrolipidbestimmungen in Leberpunktaten. Z. ges. inn. Med., **25,** 300—304 (1970).
8. Kalk, H.: Über die Fettleber des Menschen mit besonderer Berücksichtigung des Diabetes. Schweiz. med. Wschr., **89,** 1117—1121 (1959).
9. Kremer, G. J.: Qualitative Lipoidbestimmungen in pathologischen Lebern. Klin. Wschr., **46,** 109—110 (1968).
10. Kremer, G. J., Kössling, F. K., Lange, H. J., Victor, N.: Bestimmung des Fettgehalts in der Leber. Dtsch. med. Wschr., **94,** 163—169 (1969).
11. Kwiterowich, P. O., Jr., Sloan, H. R., Fredrickson, D. S.: Glycolipids and other lipid constituents of normal human liver. J. Lipid Res., **11,** 322—330 (1970).
12. Leevy, C. M.: Medicine (cit. Szarvas, F. et al. [18]) **41,** 249 (1962).
13. Magyar, I., Fischer, A.: A máj és az epeutak. Akadémiai Kiadó, Budapest p. 329 (1956).
14. Poggi, M., Di Luzio, N. R.: The role of liver and adipose tissue in the pathogenesis of the ethanol-induced fatty liver. J. Lipid Res., **5,** 436—441 (1964).
15. Postma, T., Stroes, J. A. P.: Lipid screening in clinical chemistry. Clin. chim. Acta, **22,** 569—578 (1968).
16. Rappoport, F., Eichhorn, F.: Clin. chim. Acta, 1960, **5,** 161. Cit. Bálint, P.: Klinikai Laboratóriumi Diagnosztika. Medicina, Budapest p. 446—447 (1962).
17. Saifer, A., Feldman, N.: The photometric determination of gangliosides with the sulphophospho-vanillin reaction. J. Lipid Res., **12,** 112—115 (1971).
18. Szarvas, F., Hódi, M., Tiszai, A., Kovács, K.: Über die Fettleber. Med. Klin., **59,** 648—650 (1964).
19. Youngburg, G. E., Youngburg, M. V.: Phosphorous metabolism. I. A system of blood phosphorous analysis. J. Lab. clin. Med., **16,** 158—166 (1930).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (1—2), pp. 51—57 (1974)

UTILIZATION OF IODINE KINETIC COEFFICIENTS FOR THE ANALYSIS OF PHARMACOLOGICAL RESPONSES

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(Received September 29, 1973)

A three-compartment model was designed for the analysis of drugs acting directly on thyroid function. The values for the coefficients of iodine kinetics (α, η, σ) were estimated in laboratory animals, and the responses reflected by these parameters were considered characteristic of the drugs under study. They included TSH, thyroxine and tri-iodothyronine as stimulators, and di-iodotyrosine, thiamazole and potassium perchlorate as inhibitors, of thyroid function.

TSH was found to modify iodine turnover in a direction corresponding to data in the literature, the extent of the changes being dependent upon the mode of administration. Thyroxine was found less active than expected, and the effect of tri-iodothyronine was opposed to the expected response. These observations are interpreted by a negative feed-back mechanism on the one hand, and by the possibility, on the other, that the drugs administered may not pass through all phases of iodine turnover, therefore, if the mechanism of action of an individual drug is directed at these very phases, it fails to elicit any response when attaining the blood stream.

On the evidence of the results the present method seems to be suited for the selection of appropriate drug therapy for the restoration of iodine turnover.

Drugs, including those affecting iodine metabolism, act directly or indirectly. In disturbances of iodine metabolism, direct-acting drugs are usually employed for restoration of the pharmacokinetic equilibrium. For a proper selection of the drug in a given case it is important to know which phase of iodine metabolism is affected by the individual component.

The kinetic graphs characteristic of iodine metabolism and the coefficients derived from them [8, 9, 4] allow to identify the sites of action of the individual drugs and to define their effects on a quantitative basis [2, 3].

Description and significance of the model (Fig. 1). The three-compartment model seen in Fig. 1 represents the three most currently employed coefficients.

The individual compartments of the model represent the urine, the blood and the thyroid. The coefficients α, η and σ occupy the junctions between the respective compartments. The model presupposes a migration of the substances from blood to urine and to the thyroid on the one hand, and from the thyroid to the blood, on the other. U represents the radio-iodine content of urine; S, of plasma; T, of the thyroid. The velocity coefficients α, σ and η measure the rate of iodine uptake, mobilization (secretion) and excretion, respectively.

The mathematical description of the model, in the terms of the denotations presented in Fig. 1, suits the following differential equations:

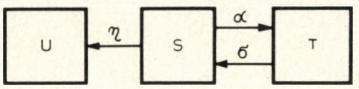

Fig. 1. Three-compartment model of iodine kinetics. T = radio-iodine content of thyroid, S = of blood, U = of urine, α = coefficient of iodine uptake, σ = of iodine secretion, η = of iodine excretion

$$\frac{dU}{dt} = \eta \cdot S \tag{1}$$

$$\frac{dS}{dt} = -(\alpha + \eta) S + \sigma T \tag{2}$$

$$\frac{dT}{dt} = \alpha \cdot S - \sigma T \tag{3}$$

The equation system also expresses the kinetics of radioactive iodine and gives the following correlations for the active iodine content of the thyroid

$$T = A\left[e^{-\sigma t} - e^{-(\alpha+\eta)t}\right] \tag{4}$$

The iodine kinetic coefficients are defined by two experimental graphs which are constructed by measurement of active iodine in blood, thyroid and urine, without differentiation of the individual iodine fractions (Fig. 2). A typical iodine retention curve of this kind is shown in Fig. 2, where T represents the changes in radioiodine content versus time for the thyroid, S, for serum; and U, for urine. From two curves constructed on this basis, the third curve can be derived in every case. Only the three significant sites of radioiodine retention have been considered, the radioiodine demonstrable at other sites of the body having been disregarded.

T_a and T_b, i.e. the respective half-lives reflected in the mobilization phase beyond 24 hr of the iodine uptake curve (T) and in the initial phase of declining serum activity (S) give the following correlations with the iodine kinetic coefficients:

$$\delta = \frac{0.693}{T_a} \quad \text{and} \quad (\alpha + \eta) = \frac{0.693}{T_b} \tag{5}$$

These two equations being given, the iodine kinetic coefficients can be computed with the aid of equation [4].

The results of studies by EICHHORN [5], JOYET and GAUTIER [6] and KUTKA and LIČKO [7] point to definite relationships between the severity of the disturbances of iodine metabolism demonstrable by clinical procedures

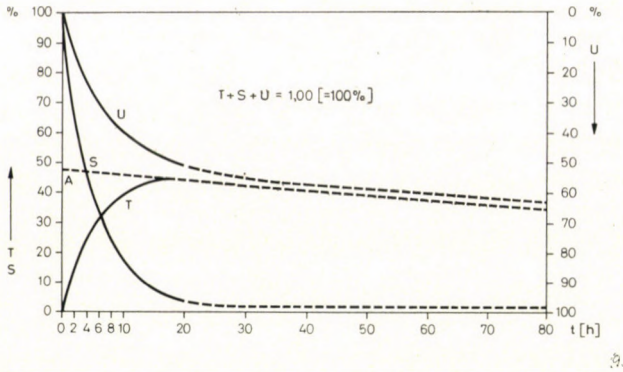

Fig. 2. Iodine kinetic curves. *T* represents the changes in the radio-iodine content of thyroid, *S* in that of blood, *U* that of urine, plotted against time. Inverse scale on ordinate

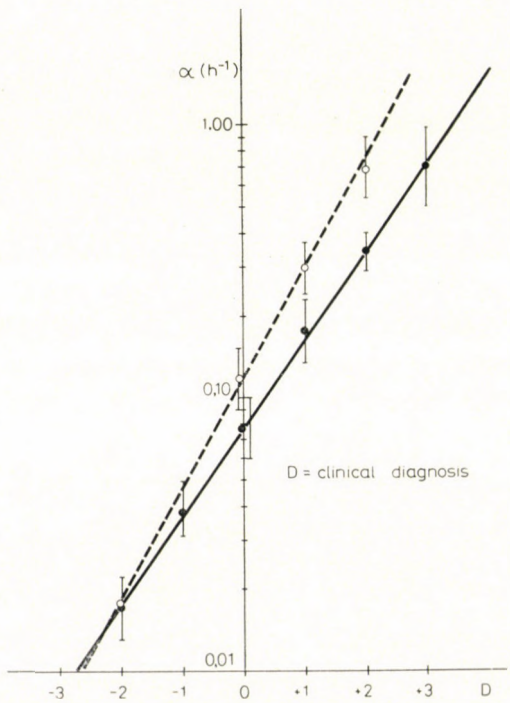

Fig. 3. Graphs representing the exponential correlations between the grade of iodine turnover disturbance established clinically, and the coefficient of iodine uptake. Log scale on ordinate

(physical examination, BMR, laboratory findings) and the coefficients of iodine kinetics. This is illustrated by the graph in Fig. 3, representing the correlations between the respective values and the severity of hyper and hypothyroidism graded from —3 to +3 (i.e. of minor, moderate and major severity). The continuous line in the graph has been derived from the data of Czechoslovakian, the interrupted line from those of Swiss authors. The graph shows that grade 0 corresponds from $\alpha = 0.08$ to 0.12 per hr, i.e. to the range characteristic of normal iodine metabolism. In a semilogarithmic coordinate system the correlations give a straight line, in other words, the relationship between the coefficient of iodine uptake and clinical severity is exponential. The other two iodine kinetic coefficients behave in the same manner.

On the grounds of the correlations seen in Fig. 3, measurement of α permits a quantitative estimation of spontaneous or drug-induced changes in thyroid function.

Material and method

Albino male rats of 200 g average body weight were used. They were kept on Remington's iodine-deficient diet for a few weeks before the experiments. The drugs acting directly on the thyroid included TSH, thyroxine (T_4) and tri-iodothyronine (T_3) as stimulators, and di-iodotyrosine (T_2) and Thiamazole as inhibitors, of iodine metabolism. The procedure has been described earlier [4].

The parameters necessary for estimation of the kinetic coefficients, i.e. the counting rates defining the radioiodine level in the thyroid and the tail vein, were estimated 2, 5, 24 and 48 hrs after administration of ^{131}I, both activities being measured simultaneously by means of a scintillation detector. The system of differential equations describing the model were solved by means of a Hewlett-Packard 9100 B computer. In this manner the kinetic coefficients and their deviations could be computed on the basis of these equations without difficulty and with high accuracy.

1. After the preparatory period referred to above, three groups of 40 animals each were formed. 30 animals of each group were treated intraperitoneally with TSH, T_4 and T_3 respectively. The other animals served as controls and received physiological saline intraperitoneally under identical conditions. In this manner the stress involved by the treatment was identical in the treated and the untreated animals. Within each group, three subgroups of 10 animals each were formed.

The animals of subgroup I were pretreated with the respective drug for a period during which it is expected to reach its maximum effect [1, 10], this period being 24 hrs for TSH and 72 hours for T_4 and T_3. The dose was 1 IU/kg daily for TSH, 1 mg/kg for T_4 and T_3.

The animals of subgroup II received the respective drug together with ^{131}I on a single occasion, in the same doses as in subgroup I.

In subgroup III, Thiamazole was administered in doses of 10 mg/kg daily for three days in order to induce hypothyroidism. This was followed by the administration of the drugs in the same doses as in subgroups I and II.

2. Three groups received T_2, Thiamazole, and potassium perchlorate, respectively. The iodine kinetic coefficients were determined in each group in 20 test animals and in 20 controls. They were administered in a preliminary as well as in an instantaneous single-dose form, the former type to subgroup I, the latter to subgroup II. Pretreatment of 4 days comprized daily doses of 60 mg/kg for T_2, 40 mg/kg for Thiamazole, and 600 mg/kg for potassium perchlorate, administered in four fractions. In the instantaneous type of treatment the same doses were applied on a single occasion.

Results and discussion

The responses are seen in Tables I and II including the iodine kinetic coefficients as well as the severity of the disturbance of iodine metabolism

Table I

Iodine kinetic coefficients under the effect of iodine turnover stimulating drugs

TSH	$\alpha(h^{-1})$	$\sigma(h^{-1})$	$\eta(h^{-1})$	A(%)	D
I.	0.2335 ±0.0300	0.0154 ±0.0020	0.391 ±0.0050	42.90	+1.35
II.	0.1980 ±0.0500	0.0078 ±0.0040	0.0183 ±0.0840	65.28	+1.20
III.	0.1970 ±0.0400	0.0090 ±0.0030	0.0260 ±0.0080	54.56	+1.20
Thyroxine (T_4)					
I.	0.1680 ±0.0400	0.0076 ±0.0030	0.0530 ±0.0010	12.96	+1.00
II.	0.1390 ±0.020	0.0022 ±0.0050	0.0300 ±0.005	33.28	+0.72
III.	0.3980 ±0.020	0.0029 ±0.0009	0.0237 ±0.0080	65.31	+2.20
Tri-iodothyronine (T_3)					
I.	0.0580 ±0.0100	0.0130 ±0.0020	0.0440 ±0.0100	15.72	−0.35
II.	0.1280 ±0.030	0.0077 ±0.0010	0.0250 ±0.0070	42.72	+0.65
III.	0.0270 ±0,0090	0.0408 ±0,0010	0.0802 ±0.0100	6.40	−1.40
Controls					
	0.0850 ±0.0100	0.0051 ±0.0010	0.0800 ±0.0100	45.5	±0.20

which is presented in the last column. A represents the figure for the mobilization phase ($\sim e^{-\delta t}$) of the iodine-retention curve extrapolated back to the time of administration. Its value, in addition to the kinetic coefficients, may be characteristic of the response elicited by the drug.

The results set out in Table I show that TSH, whether administered as pretreatment or in a single dose, enhanced iodine turnover. On the other hand, T_3 which is usually considered more active than thyroxine, produced a slight increase in thyroid function when administered in a single dose and had an opposite effect when applied in the form of pretreatment. This may have the following explanation.

Table II

Iodine kinetic coefficients under the effect of iodine turnover inhibiting drugs

Di-iodothyrosine (T_2)	α (h^{-1})	σ (h^{-1})	η (h^{-1})	A %	D
I.	0.013 ±0.005	0.00079 ±0.00010	0.129 ±0.020	20.30	−2.4
II.	0.022 ±0.008	0.00183 ±0.00070	0.099 ±0.004	20.94	−1.7
Thiamazole					
I.	0.035 ±0.010	0.0035 ±0.0006	0.0265 ±0.0100	35.10	−1.05
II.	0.093 ±0.008	0.00151 ±0.0004	0.0284 ±0.0050	41.10	+0.20
Potassium perchlorate					
I.	0.026 ±0.003	0.00036 ±0.00110	0.0541 ±0.0030	12.29	−1.45
II.	0.013 ±0.002	0.00260 ±0.00070	0.0520 ±0.0100	− 2.61	−2.4
Controls					
	0.085 ±0.010	0.00510 ±0.00100	0.0800 ±0.0100	45.5	+0.2

If T_3 is conveyed to the blood stream in a single dose, it may miss the phase of iodine turnover which is essential to its mechanism of action, thus being prevented from exerting its stimulating activity.

On the other hand, the depressive effect of T_3 on thyroid function in the case of sustained treatment or of administration of thyrostatic drugs, may be attributed to a negative feed-back mechanism. In the present case, the negative feed-back between trophic hormone and target hormone (TSH —T_3) signifies that a rise or fall in the blood thyroid hormone level results in an opposite change of TSH secretion, moreover, it is to be assumed that by the administration of exogenous thyroid hormone a direct inhibitory mechanism is also made to act on thyroid secretion. The observation that the stimulating action of exogenous thyroid hormone on iodine turnover is weaker in euthyroid animals than in those of subgroup III which have been rendered hypothyroid by Thiamazole pretreatment is presumably also the result of the same feed-back mechanism. Human observations also bear testimony to the suppressive effect of thyroxine.

On the evidence of the data in Table II, all three thyrostatic drugs under study exhibited a distinct and significant inhibitory action. It is only the degree of efficiency which differed according to the mechanism of action of the drug in question and to its mode of administration.

The considerable fall in iodine turnover consequent upon a single dose of T_2 was still more marked after prolonged administration. It may be assumed that iodine being split off from the massive amounts of T_2 exerts a direct inhibitory effect on thyroid function.

The effect of Thiamazole is due to the fact that all mercaptoimidazole derivatives are inhibitory to the peroxidase enzyme bringing about the oxidation of iodine to iodide. As a result, inorganic iodide taken up by the thyroid accumulates there without being utilized for biosynthesis and interferes with the uptake of further inorganic iodide. As it can be seen from Table II, Thiamazole has to be administered for a certain time to ensure an optimum effect.

The perchlorate ion interferes with iodine uptake by a competitive inhibition, therefore even a single dose of it is causing a significant reduction of the kinetic coefficients.

Some of the drugs studied did not affect the individual kinetic coefficients to the same extent. For instance, the considerable reduction in σ produced by Thiamazole was not necessarily followed by a fall in α of the same order.

The results show that in the diverse disturbances of iodine turnover the various drugs are not of equal benefit. It is the extent, direction and site of action of these drugs which the present findings might help to assess, thus allowing to prescribe the treatment correctly corresponding to the prevailing disturbance of iodine turnover.

REFERENCES

1. Bálint, P.: Orvosi élettan. Medicina, Budapest p. 542 (1972).
2. Bartha, K. G.: Acta med. **25,** 121 (1968).
3. Bartha, K. G.: Acta med. **28,** 271 (1971).
4. Bartha, K. G., Kanyár, B.: Kísérl. Orvostud. **24,** 65 (1972).
5. Eichhorn, O.: Schweiz. med. Wschr. **85,** 897 (1955).
6. Joyet, G., Gautier, R.: Bull. Schweiz. Akad. med. Wiss. **11,** 82 (1955).
7. Kutka, M., Ličko, V.: Bratisl. lek. Listy **42,** 334 (1962).
8. Kutka, M., Ličko, V.: Naturally Occurring Goitrogens and Thyroid Function. Publishing House of the Slovak Academy of Sciences, Bratislava p. 141(1964).
9. Kutka, M., Németh, S.: Endokr. pol. **16,** 493 (1965).
10. Pitt-Rivers, R., Trotter, W. R.: The Thyroid Gland. Butterworths, London 199, 1964, Vol. 1. P. 199.

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (1—2), pp. 59—67 (1974)

RENAL FUNCTION AND MORPHOLOGY IN HYPERTENSION INDUCED BY LIGATION OF ONE RENAL ARTERY IN THE RAT

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(Received October 4, 1973)

Renal function and morphology were studied in hypertension induced in rats by ligation of one renal artery without interference with the other kidney. 13 and 30 weeks after the intervention when blood pressure and renal hypertrophy were considered to be in a steady state, renal function displayed certain changes. Simultaneously, vascular lesions of hypertensive character were demonstrated in the unoperated kidney. The role of hypertrophy in the maintenance of normal blood pressure and renal function, and the role of renovascular degeneration in the pathomechanism of hypertension and renal dysfunction are discussed.

As reported earlier (FEKETE and TARJÁN [12]), we have been able to induce a state of permanent hypertension in rats by ligation of one renal artery without interference with the other kidney. In opposition to other experimental methods making use of adjustable clamps and involving, by this fact, individual differences of renal blood flow, the procedure applied allowed entirely to block the blood supply to one kidney and to study the state and function of the other, unmanipulated, kidney as long as necessary.

In order to study whether the procedure was suitable for the experimental study of human renovascular hypertension, serial experiments concerned with the features of the hypertensive state thus produced have been undertaken. These studies revealed 1. a slight elevation of blood pressure between the 2nd and 6th week after the intervention; 2. a significant elevation of blood pressure and its subsequent stabilization at high levels between the 13th and 30th week; 3. hypertrophy of the heart and of the unoperated kidney with reference to body weight, the peak values being demonstrable before blood pressure had attained a high level, i.e. between the 2nd and 6th week.

Human hypertensive conditions are associated with characteristic modifications of certain, primarily renal, mechanisms. The functional aspects of experimental renal hypertension have thus far received little attention. In the experiments to be reported the individual parameters of renal function and the morphology of the kidney have been studied 13 and 30 weeks after ligation of one renal artery, at a stage when arterial blood pressure and renal hypertrophy have reached a steady state.

Material and method

Home-bred two-month-old female albino rats with a mean weight of 180 g were used. They were fed on synthetic rat chow containing 150 mEq/kg of sodium. In 25 animals under ether anaesthesia, the region of the left kidney was exposed from the midline approach and the left renal artery was ligated without complete isolation, then the wound was closed.

At 13 and 30 weeks after the intervention the animals were divided into two groups of 13 and 10 rats, respectively, then weighed and placed in individual metabolism cages. Their urine was collected for 7 hours daily for 3 to 4 days, invariably between 9 a. m. and 4 p. m. Water was allowed ad libitum. At the end of these periods, under 30 mg/kg pentobarbital anaesthesia arterial blood pressure was measured with a mercury manometer inserted into the carotid of one side, then blood samples were withdrawn from the carotid, and the heart, both kidneys, and the gut with the mesenterium were removed, weighed and stored for processing.

Chemical analysis. The pooled urine was measured daily and the urinary and blood samples were studied for osmolality with a Fiske osmometer, for sodium and potassium concentrations with a Zeiss flame-photometer and for creatinine concentrations by the method of BÁLINT and VISY [1].

Control group. Twelve unoperated female rats with 227 g mean weight and correspondrng in age to the hypertensive ones were handled in the same manner as described above, inoluding urine-collections, blood-pressure-readings, samplings, etc. and in 5 animals, the same cigans as in the test group were studied histologically.

Statistical analysis was done by Student's two-sample *t*-test.

Histology. The organs were fixed in 10% formalin embedded in paraffin, stained with haematoxylin-eosin, van Gieson's and Mallory's phosphotungstic acid haematoxylin.

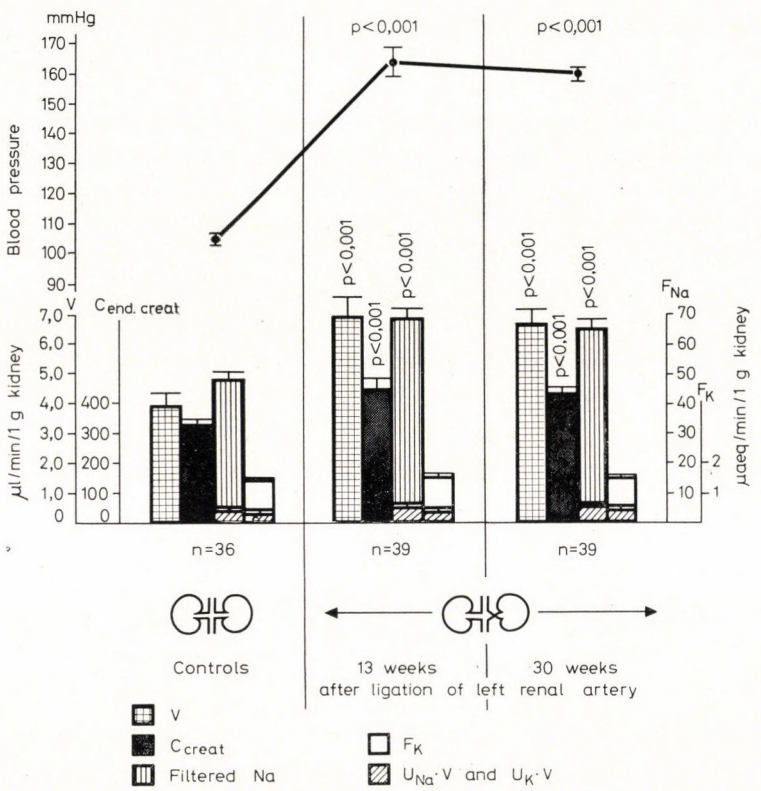

Fig. 1. Parameters of renal function and blood pressure in rats, 13 and 30 weeks after ligation of one renal artery, as compared with the controls

Results

Results are shown in Table I and Fig. 1.

Table I

Parameters of renal function in rats after ligation of the left renal artery and in normal controls ($\bar{x} \pm s_{\bar{x}}$)

Parameters	Control groups	13 weeks after ligation of left renal artery	30 weeks after ligation of left renal artery
Number of animals	12	13	10
Weight of animals, g	227±12	253±8	272±8
Weight of right kidney, mg	806±3	1357±51[d]	1431±98[d]
Weight of left kidney, mg	803±3	182±8[d]	149±7[d]
Weight of right kidney in per cents of body weight	0.36±0.01	0.53±0.02[d]	0.53±0.05[d]
Weight of left kidney in per cents of body weight	0.36±0.01	0.07±0.01[d]	0.05±0.01[c]
Blood pressure, mmHg	105±2	164±5[d]	160±2[d]
V μl/min/l g kidney	3.94±0.37	6.91±0.73[d]	6.71±0.45[d]
U_{osm}/P_{osm}	5.40±0.23	2.77±0.23[d]	—
S_{creat}, mg/100 ml	0.82±0.04	0.93±0.06	0.95±0.04
C_{creat}, μl/min/l g kidney	329±21	456±0.26[d]	435±20[d]
S_{Na} mEq/l	147±2	150±2	150±1
$C_{creat} \cdot S_{Na}$ μEq/min/l g kidney	48.4±3.1	68.5±3.7[d]	65.3±2.9[d]
$U_{Na} \cdot V$ μEq/min/l g kidney	0.416±0.06	0.518±0.06	0.463±0.04
Excreted Na/filtered Na, %	0.86±0.12	0.76±0.0[p]	0.71±0.06
S_K mEq/l	4.5±0.1	3.4±0.1[c]	3.5±0.03[c]
$C_{creat} \cdot S_K$ μEq/min/l g kidney	1.46±0.05	1.57±0.10	1.52±0.08
$U_K \cdot V$ μEq/min/l kidney	0.38±0.05	0.42±0.03	0.47±0.03
Excreted K/filtered K, %	26.1±2.4	25.6±0.8	30.9±4.3
Haematocrit, per cent	43±2	40±1	41±1
Number of measurements (urine)	36	39	39
Number of measurements (serum)	12	13	10

Symbols: c = $p < 0.01$; d = $p < 0.001$

1. As pointed out in earlier studies, ligation of one renal artery in rats is followed by a statistically significant elevation of blood pressure with a 55 mmHg mean rise by 13 and 30 weeks. The control mean values were 105±2 mmHg and by 13 and 30 weeks, 164±6 and 160±2 mmHg, respectively.

2. At 13 and 30 weeks after ligation of the left renal artery, the ischaemic kidney showed marked atrophy, its function was negligible, in contrast to the

unoperated kidney which displayed a significant hypertrophy, its weight having increased from 0.36 to 0.53% of the body weight. The parameters of renal function were referred to 1 g weight of the unoperated kidney and the values thus expressed correlated to those of the control group (Fig. 1).

a) Urinary output per minute rose by approximately 70%. Urinary osmolarity showed a parallel decline, so the ratio U_{osm}/P_{osm} diminished to approximately 50% of its original value.

b) Endogenous creatinine clearance displayed a significant increase of approximately 40%. The serum creatinine level was unaffected.

c) The serum sodium level remained unchanged as compared with the control. As a result of the increased GFR, the amount of filtered Na increased by approximately 40%. As natriuresis increased only by 20%, excretion of Na in per cents of the filtered load fell from 0.9 to 0.7%. The change was, however, not significant statistically.

d) The serum potassium level showed a significant decrease. The filtered amounts of potassium thus remained unchanged while its excretion displayed a slight increase to 30% of the filtered load. The changes were at the borderline of significance.

Microscopic study. In 5 control animals, the microscopic structure of the kidney was normal. In one animal there was a slight lymphocytic infiltration of focal character in the cortical interstitium. No change was demonstrable in the renal arteries and arterioles.

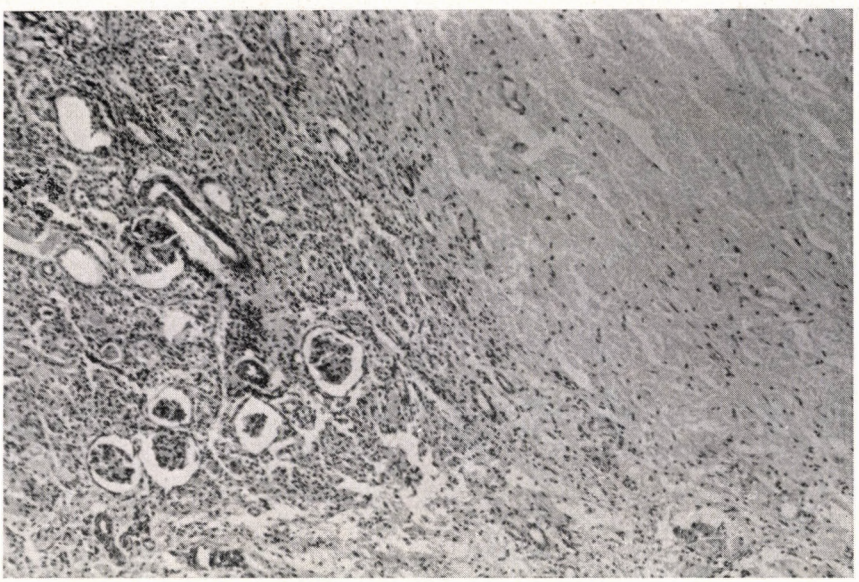

Fig. 2. Microscopic appearance of the kidney 3 weeks after arterial ligation. Extensive necrosis side by side with a fairly preserved, atrophied cortical area. H—E, ×50

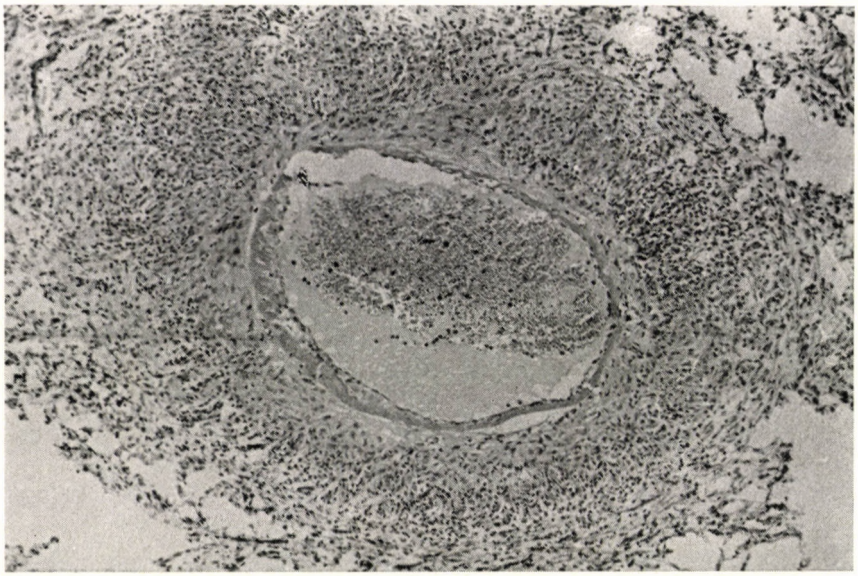

Fig. 3. Mesenteric artery of an animal subjected to ligation of one renal artery 27 weeks before. Extensive fibrinoid necrosis of intimal and medial layers, massive inflammatory granulation involving the adventitia. H—E, ×80

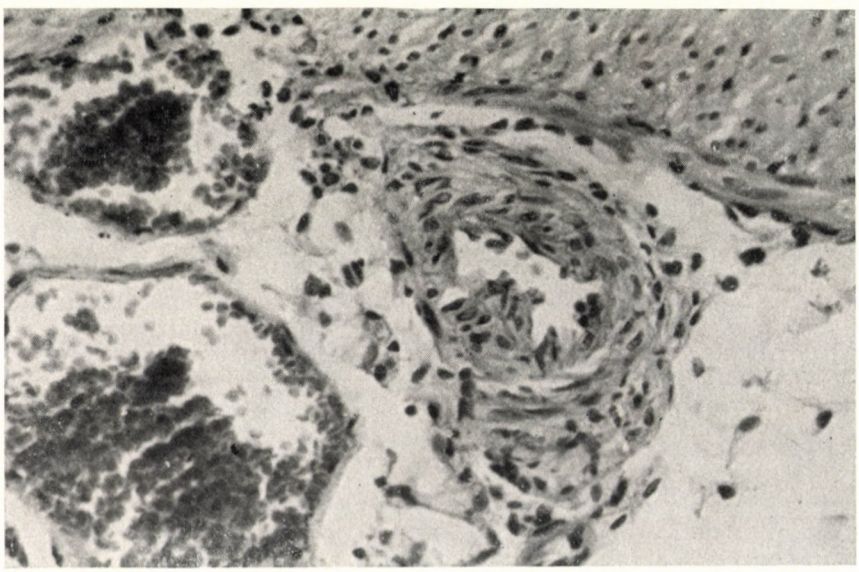

Fig. 4. Arteriole from the subserosa of the large intestine 13 weeks after ligation of one renal artery. Proliferation of intima, broadening of muscular layer, granulation and scarring of adventitial layer. H—E, ×350

In the hypertensive animals, the kidney with its artery ligated displayed extensive necrotic areas with patchy calcified deposits, and atrophic areas with signs of tubular regeneration, demarcated from the necrotic areas by scar tissue (Fig. 2). Vascular changes confined to moderate thickening and fibrosis were found in a single case.

The unmanipulated kidney showed microscopic signs of hypertrophy (enlargement of tubular epithelium, moderately enlarged glomeruli).

Vascular changes in the heart, gut and, in 16 cases in the kidney were demonstrable in 18 of the 21 animals. In 4 cases, these changes consisted of fibrinoid necrosis or of polyarteritis nodosa typical of malignant hypertension in albino rats (Fig. 3). The pattern was, however, generally that of hypertension of moderate severity characterized by hypertrophy of the media and by hyperplasia of the intima (Fig. 4). These changes appeared generally 5 to 6 weeks after the arterial ligation and their severity was related to the degree and duration of the hypertension (Fig. 5).

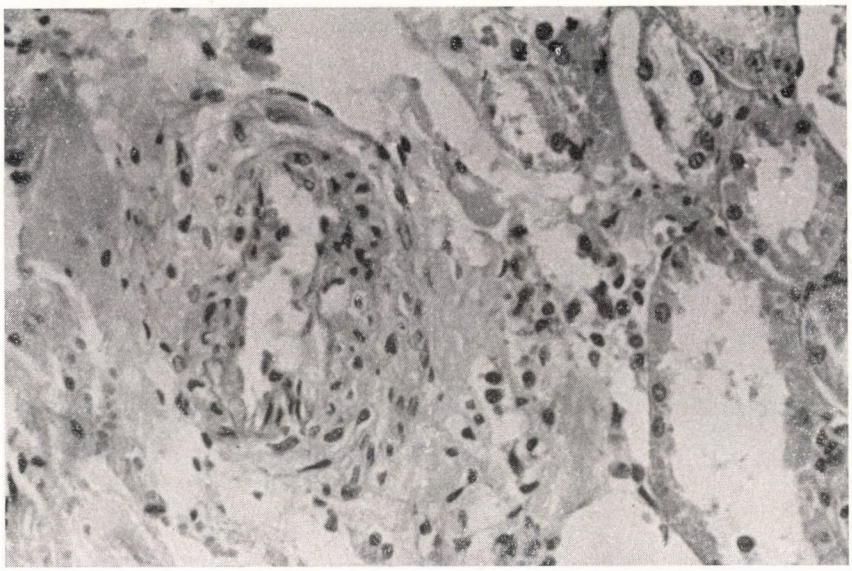

Fig. 5. Artery from unoperated kidney 9 weeks after ligation of the contralateral renal artery. Changes like in Fig. 4. H—E, ×350

Discussion

The relationships between kidney and arterial hypertension were analyzed by WILSON [36] on the grounds of experimental evidence including his own studies. In these, a narrowing of one renal artery had been produced without interference with the other kidney and the hypertension thus induced usually persisted after removal of the ischaemic kidney. The failure to nor-

malize blood pressure was attributed to vascular lesions in the unmanipulated kidney, regarded as a direct consequence of the hypertension.

In the rat experiments by FLOYER [13], unilateral renal arterial constriction was followed by an acute elevation of blood pressure which then stabilized in the course of a few weeks. Removal of the ischaemic kidney within this period was followed by normalization of blood pressure, the unoperated kidney having been found unaffected or its changes reversible. The stabilization of hypertension is due to the involvement of the unmanipulated kidney caused by a vasoconstrictive factor produced in the ischaemic kidney. At this stage, beyond the 5th to 7th week, removal of the ischaemic kidney does not any more lead to a normalization of blood pressure since with the progress of vascular lesions the unoperated kidney gradually loses its capacity of inhibiting the pressor substances. As it has been formulated by SELYE [34] and by MASSON et al. [24] the kidney, devoid of blood supply, is transformed into an endocrine kidney.

Information is scarce concerning the functional parameters associated with experimental renal hypertension in the rat. We find only short-term observations in the literature and the experimental procedures are inconsistent. It is, however, certain that at 2 to 3 weeks after the intervention causing hypertension, GFR and Na-reabsorption in the unmanipulated kidney may attain 88% of the control figures (MALT [25]) and that there is a 7 to 28% increase in inulin clearance from the normal 0.35 ml per 100 g body weight at 2 weeks (DICKER and SHIRLEY 1971). HAYSLETT et al. [15] demonstrated in individual nephrons a 30% increase in GFR and in the filtered as well as the reabsorbed amounts of Na. MAXWELL [28], PEART [31], DEL GRECO et al. [7] and CONN et al. [6] noted hypopotassaemia in association with hypertension.

The present observations extending to 30 weeks after ligation of one renal artery revealed in the presence of the unmanipulated opposite kidney 1. a gradual and significant elevation of blood pressure; 2. a hypertrophy, and 3. functional as well as morphological changes in the unoperated kidney.

The mechanism of compensatory hypertrophy is not clear (MERTZ and WEISS [29]). Hypertrophy of the renal tissue represents a response either to the loss of functional capacity or to deviations from the ideal mass of renal tissue (DICKER [9]). MALT [25] ascribes the renal hypertrophy to the incremental work load. Specific control mechanisms of some kind have been also suggested to have a role (PHILLIPS and LEONG [30]). As regards the time-relationships, the process begins in the very first hours (COE and KORTY [5]; JANITZKY and LINGIS [18]) and may attain a maximum of 70 to 80% by the 20th day (SAPHIR [33]; ROLLASON [32]).

The antihypertensive function of intact renal tissue has been given different interpretations. BRAUN-MENENDEZ [3] regards renotrophin as the

stimulator of renal growth. PEART [31] attributes the elevation of blood pressure to an abnormal breakdown of pressor substances, MASSON [23] to a deficient production of antidepressor material. SEN et al. [35] as well as DEVAUX et al. [8] incriminate a decrease in the amount of renin-inhibitor. In experimental hypertension induced in dogs by ligation of one renal artery (FEKETE et al. [11]) the unmanipulated kidney showed no hypertrophy while being affected by degenerative vascular changes, and elimination of the ischaemic kidney failed to cause a normalization of blood pressure. In patients in whom surgical removal of the ischaemic kidney failed to decrease or to normalize the high blood pressure, the renal and extrarenal arterioles revealed degenerative vascular lesions typical of hypertension (MCCORMACK [27]; HUBER [16]; KOLESTKY and RIVERA-VELEZ [21]; MCALLISTER et al. [26]; etc.).

In the present experiments the morphological changes took 5 to 6 weeks to develop. The microscopic features of malignant hypertension thus induced were consistent with those found in other model experiments. The light and electron microscopic features and the mechanism of hypertensive vascular changes have widely been studied (ENDES [10]; CHURG [4]; KERÉNYI et al. [20]; JELLINEK et al. [19]; HÜTTNER et al. [17]; KONYÁR et al. [22]). In the animals which had remained free from necrotic lesions, vascular changes of hyperplastic, proliferative character were prevalent, owing probably to the vascular injury having been moderate and so the increase in blood pressure, gradual (GOLDBLATT [38]; ZOLLINGER [37]; HATT et al. [14]).

Our observations lend support to the claim that after ligation of one renal artery in rats the unoperated kidney undergoes hypertrophy, and becomes capable of maintaining blood pressure at normal levels and takes over the function of the ischaemic kidney. This compensatory mechanism, ideal though it seems, is of a transitory nature, remaining in operation for a period not longer than 4 weeks. Its breakdown which is bound to ensue 5 to 6 weeks after the arterial ligation is in all probability due to an enhanced production of pressor substances by the ischaemic kidney and to the consecutive vascular lesions in the unoperated kidney. This process is then characterized by significant elevation of blood pressure and the impairment of tubular function with the GFR unaffected.

Acknowledgement

We are indebted to Mrs. Á. SPITZÁR for skilful technical assistance.

REFERENCES

1. BÁLINT, P., VISY, M.: Kísérl. Orvostud., **17,** 326 (1965).
2. BOHLE, K.: Virchows Arch. path. Anat. **323,** 1 (1953).
3. BRAUN-MENENDEZ, E.: Circulation **17,** 696 (1958).
4. CHURG, J.: Arch. Path., **75,** 547 (1963).

5. Coe, F. L., Korty, P. R.: Amer. J. Physiol., **213,** 1585 (1967).
6. Conn, J. W., Rovner, D. R., Cohen, E. L.: Ann. intern. Med., **63,** 266 (1965).
7. Del Greco, F., Simon, N. M., Goodman, S., Roguska, J.: Medicina **46,** 475 (1967).
8. Devaux, C., Meyer, P., Idatte, J. M., Milliez, P.: Nephron **6,** 612 (1969).
9. Dicker, S. E.: J. Physiol. (Lond.) **225,** 577 (1972).
10. Endes, P.: Acta morph. Acad. Sci. hung. **14,** 307 (1966).
11. Fekete, A., Forgács, I., Gaál, K., Mészáros, T.: Acta med. Acad. Sci. Hung. **28,** 181 (1971).
12. Fekete, A., Tarján, É.: Acta med. Acad. Sci. Hung. **30,** 408 (1972).
13. Floyer, M. A.: Ciba Found. Symposium on Hypertension. Ed. Wolstenholme, H. E. W. Churchill, London 1954.
14. Hatt, P., Berjal, G., Bonnet, M., Jouannot, P.: Coll. int. CNRS **169,** 872 (1967).
15. Hayslett, J. P., Kashgarian, M., Epstein, F. H.: Fed. Proc. **26,** 375 (1967).
16. Huber, J.: Z. ges. exp. Med. **133,** 285 (1960).
17. Hüttner, I., Jellinek, H., Kerényi, T.: Exp. molec. Path., **9,** 303 (1968).
18. Janicky, R., Lingis, J.: Amer. J. Physiol. **219,** 1188 (1970).
19. Jellinek, H., Nagy, Z., Hüttner, I., Bálint, A., Koczé, I., Kerényi, T.: Brit. J. exp. Path., **50,** 13 (1969).
20. Kerényi, T., Jellinek, H., Hüttner, I., Gorácz Gy., Konyár, É.: Acta morph. Acad. sci. hung., **14,** 175 (1966).
21. Koletsky, S., Rivera-Velez, S. M.: J. Lab. clin. Med., **76,** 54 (1970).
22. Konyár, É., Földes, J., Jellinek, H., Krasznai, I.: Morph. Ig. Orv. Szle **11,** 195 (1971).
23. Masson, G. M.: Experientia (Basel) **18,** 243 (1962).
24. Masson, G. M., Kashii, C., Panisset, J. C.: Canad. med. Ass. J. **90,** 231 (1964).
25. Malt, R. A.: New Engl. J. Med. **280,** 1446 (1969).
26. McAllister, R. G., Michealakis, A. M., Oates, J. A., Foster, J. H.: J. Amer. med. Ass. **225,** 865 (1972).
27. McCormack, L. J.: Med. Clin. N. Amer., **45,** 247 (1961).
28. Maxwell, M. H.: Amer. J. Cardiol. **9,** 126 (1962).
29. Mertz, D. P., Weiss, M.: Dtsch. med. Wschr. **96,** 531 (1971).
30. Phillips, T. L., Leong, G. F.: Cancer Res., **27,** 286 (1967).
31. Peart, W. S.: Arch. intern. Med. **104,** 347 (1959).
32. Rollason, H. D.: Anat. Rec., **104,** 263 (1949).
33. Saphir, O.: Amer. J. Path. **3,** 329 (1927).
34. Selye, J.: J. Urol. (Baltimore) **56,** 399 (1944).
35. Sen, S., Smeby, R. R., Bumpus, F. M.: Amer. J. Physiol., **214,** 337 (1968).
36. Wilson, C.: Lancet **2,** 579 (1953).
37. Zollinger, H. V.: Schweiz. Z. allg. Path., **22,** 272 (1959).
38. Goldblatt, H.: Physiol. Rev. **27,** 120 (1947).
39. Dicker, S. E., Shirley, D. G.: J. Physiol. 210, 53 P, 1970

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (1—2), pp. 69—75 (1974)

SERUM GLYCOPROTEINS IN MYOCARDIAL INFARCTION

By

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(Received October 12, 1973)

The serum IgG, IgA, IgM, coeruloplasmin, α_2—macroglobulin and transferrin levels were estimated by the radial immunodiffusion technique and followed up over a period of 40 days in patients with acute myocardial infarction. IgG and IgA levels were elevated between the 16th and 21st days. The coeruloplasmin level showed the most marked elevation, and had not completely normalized by the 40th day. The α_2 macroglobulin level revealed a similar, though less marked, tendency. The transferrin level decreased.

The proteoglycans and the glycoproteins (GP) belong to the essential constituents of the connective tissue ground substance. Abnormalities of the ground substance almost inevitably affect the metabolism of GP, even in the absence of any primary defect. Metabolic changes of this kind may be associated with abnormal serum GP levels [1, 9, 17].

The modifications in the carbohydrate components of GP, in particular of protein-bound hexose, hexosamine, sialic acid and fucose have been studied in a wide variety of pathologic processes, including connective tissue and kidney diseases, malignant growths and other conditions. In the majority of these abnormalities an increase occurred in the levels of the carbohydrate components of GP [4, 8, 11, 17].

Human serum is known to contain numerous GP fractions which, despite their individual physical, chemical, immunological and biological characters [11, 13], have common carbohydrate components. Therefore, quantitative changes in their concentration provide no information on the individual GP fractions. Consequently, we have to study the concentrations of the different GP individually. This might offer closer information on the aetiology and pathogenesis of various processes and possibly also diagnostic clues. A study has therefore been made of the changes of GP in myocardial infarction with a view to their pathogenetic and diagnostic implications.

The values were estimated by the radial immunodiffusion technique over a period of 40 days following the attack.

Material and method

The study concerned 35 patients admitted with myocardial infarction in the period 1972 to 1973. There were 23 males, 12 females, their age ranged from 27 to 77 years (mean, 57.3 years for males, 71.6 years for females). The diagnosis of myocardial infarction was based on the history, clinical course, blood counts, ESR, ECG, serum lipid, SGOT, SGPT and SLDH levels. Serious complications including shock, pericarditis, pulmonary embolism, major disorders of cardiac rhythm, occurred in 15 patients, on 16 occasions altogether. The concentrations of IgG, IgA, IgM, coeruloplasmin, α_2-macroglobulin and transferrin were measured in native serum from the onset of the attack over a period of 40 days at seven different times by the radial immunodiffusion techique of MANCINI et al. [10], using monospecific sera (supplied by the Institute of Human Vaccine Production and Research, Budapest). Pooled human sera from 100 normal blood donors served as control. The results were quantified on the basis of reference sera (HYLAND, KALLESTAD). Since no transferrin reference sera were available, these results were referred to the generally accepted standard values given by BECKER et al. [2]. For statistical analysis, the two-sample t test was used. The results agreed with the values given by the Institute of Human Vaccine Production for IgG and IgA but showed some deviation for IgM.

Results

Results have been tabulated and represented graphically (Tabl I). IgG concentration began to rise between the 8th and the 15th day and was significantly increased between the 16th and 21st days, to return subsequently to the normal level. IgA concentration too was significantly increased between the 16th and 21st days and after a transitory elevation ($p > 0.05$) normalization ensued in the second half of the observation period. The IgM level decreased between the 1st and 3rd days.

Coeruloplasmin values began to rise in the course of the first days and were significantly elevated until the 33rd day when they began to decline. α_2–macroglobulin also showed a rising tendency from the outset; its elevation was sig-

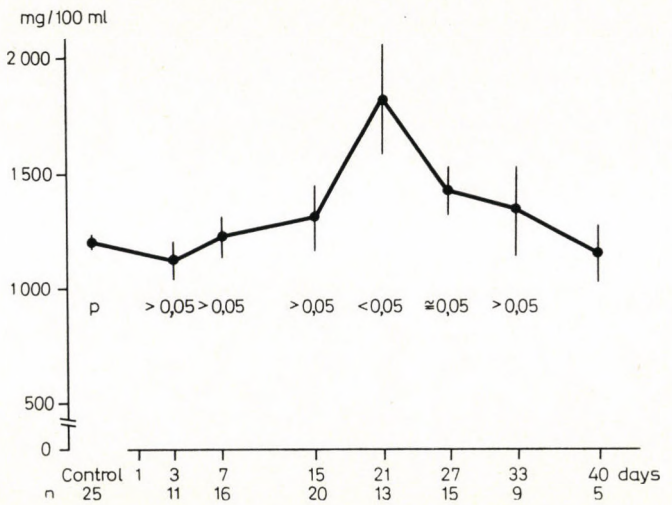

Fig. 1. The changes in the concentrations of the IgG

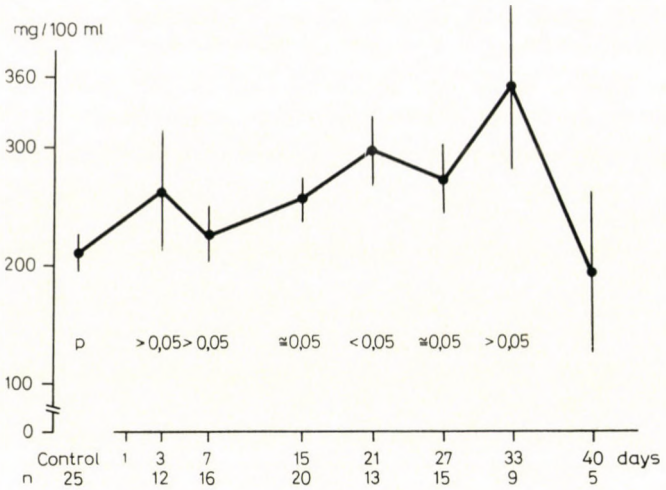

Fig. 2. The changes in the concentrations of the IgA

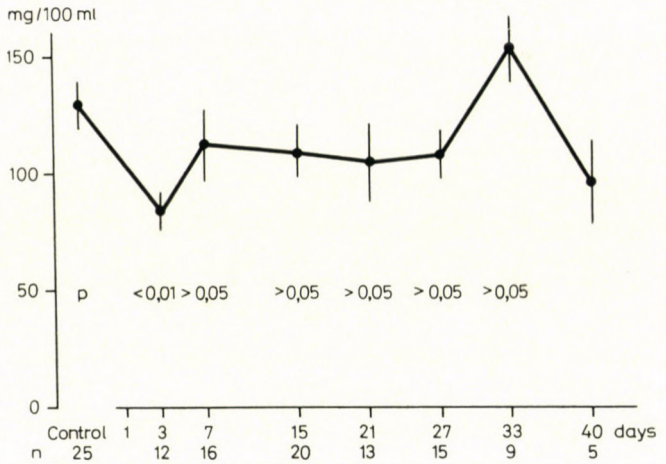

Fig. 3. The changes in the concentrations of the IgM

nificant between the 4th and 7th days and was still at the borderline of significance between the 22nd and 27th days. Transferrin displayed a characteristic pattern. Its value decreased from the very beginning and was lowest between 8 and 15 days, to rise again afterwards and to attain nearly normal values at 40 days.

The number of cases was too small for grouping the cases on the basis of complications. It is none the less to be noted that in disorders of cardiac rhythm there was a moderate increase in the concentrations of α_2-macroglobulin, and in shock, a more marked increase in IgG and IgA.

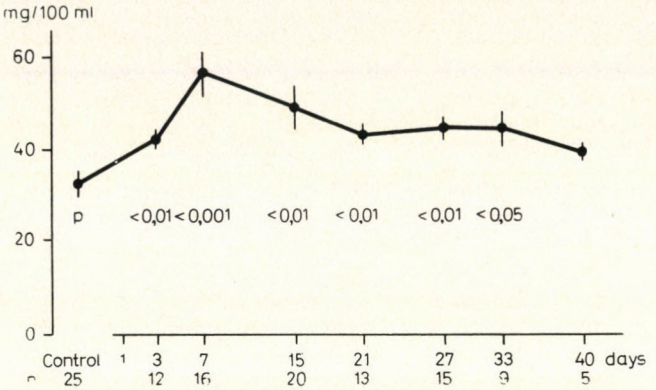

Fig. 4. The changes in the concentrations of coeruloplasmin

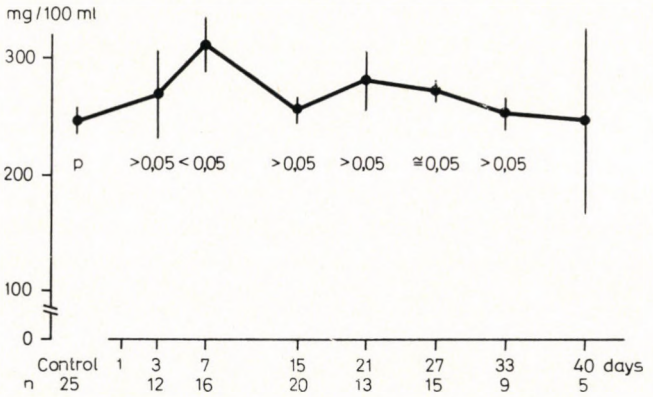

Fig. 5. The changes in the concentrations of α_2-macroglobulin

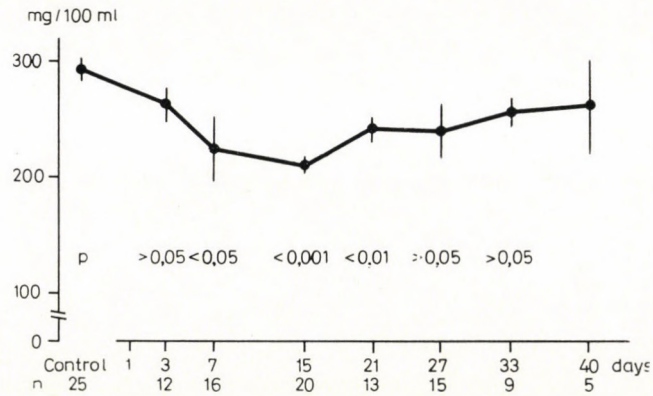

Fig. 6. The changes in the concentrations of transferrin

Discussion

Studies of the serum GP with individual biological and immunological characters have been found informative concerning their diagnostic value. CLEVE and STROHMEYER [3], studying the α_1–acid GP, α_2–macroglobulin and Gc concentrations found an increase in α_1-acid GP in cholangitis, in systemic diseases and tumours, and a decrease in cirrhosis of the liver, and a reduction in Gc-globulin level in liver disease. SNYDER and ASHWELL [14] estimated 15 different GP fractions in malignant processes against pathologic and normal controls. Though evaluation of their results is difficult by the small number of cases and their heterogenous character, the fact remains that seven of the GP fractions concerned (transferrin, α_2-macroglobulin, Gc globulin, IgA, IgD, IgG, IgM) remained unaffected, while three (α_1-acid GP, coeruloplasmin, α_1-antitrypsin) showed increased concentrations in both abnormal groups. Two fractions (haptoglobin, haemopexin) had increased in malignant disease, whereas the concentration of three ($\alpha_{2HS}{}^{-GP}$, β_2—GPI, prealbumin) reduced in malignancy and unaffected in other abnormal conditions. In the seromucoid group regarded earlier as homogeneous, increasing, unchanged and declining GP levels were equally represented. MIESCH et al. [12] practically never found a normal α_1-antitrypsin level in pathologic conditions, in contrast to the α_2-macroglobulin concentration which remained almost invariably normal, apart from cases of tumour and hepatitis where they were increased to some extent. SZÉKELY et al. [15] noted increased serum haptoglobin levels in myocardial infarction, cerebrovascular insults and peripheral arteriosclerosis. Serum haptoglobin was measured on the basis of the haemoglobin-binding capacity, and serum coeruloplasmin by means of colorimetry by HEVÉR et al. [7] and by TÓTH et al. [16]; the haptoglobin was found to increase in malignant disease parallel with the growth of the tumour and in leukaemia. An increased coeruloplasmin level was observed in malignancy of the blood-forming organs, with the exception of multiple myeloma. The serum GP was extensively studied in chronic liver disease by FEHÉR et al. [5, 6]. Coeruloplasmin and α_2-macroglobulin increased in chronic aggressive hepatitis and in cirrhosis of the liver, IgM in chronic persistent hepatitis, IgG and IgA in chronic aggressive hepatitis and in cirrhosis of the liver. ZAWADSKI and EDWARDS [18] noted hypertransferraemia in iron-deficiency anaemia. The behaviour of the serum-GP in myocardial infarction has not yet been studied by means of modern methods.

The present results raised the following points.

The GP character of immunglobulins is well-known. While it is difficult to account for the early decline of the IgM level, the increase in IgA and IgG may be connected with an immunological nature of the events. The haematocrit showed variations of no significance. Interpretation of the behaviour of transferrin awaits further evidence.

Table I

Serum-glycoprotein concentrations (mg/100 ml) in acute myocardial infarction

		IgG	IgA	IgM	Coeruloplasmin	α_2-macroglobulin	Transferrin
Controls	n	25	25	25	25	25	25
	$\bar{x}$	1200	210	130	31	244	295
	SD	56.0	16	10.6	2.2	12	11
	p						
1—3 day	n	11	12	12	12	12	12
	$\bar{x}$	1125	263	83.7	42	270	263,55
	SD	91.4	57.5	9	2.1	39.2	15.8
	p	>0.05	>0.05	<0.01	<0.01	>0.05	>0.05
4—7 day	n	16	16	16	16	16	16
	$\bar{x}$	1224	225	113	56.6	314	224
	SD	104	26.3	26.4	5.7	26.1	26.3
	p	>0.05	>0.05	>0,05	<0.001	<0.05	<0.05
8—15 day	n	20	20	20	20	20	20
	$\bar{x}$	1308	255.6	109.9	49	258	212
	SD	147	20.8	57.1	5.9	12.2	5.7
	p	>0.05	≅0.05	>0.05	<0.01	>0.05	<0.01
16—21 day	n	13	13	13	13	13	13
	$\bar{x}$	1818	298	105.5	43	282	244
	SD	250	31.5	18.5	2.9	27.7	12.3
	p	<0.05	<0.05	>0.05	<0.01	>0.05	<0.01
22—27 day	n	15	15	15	15	15	15
	$\bar{x}$	1421	223	108	44.4	275	242
	SD	114	29.7	11.4	3	10.8	26
	p	≅0.05	≅0.05	>0.05	<0.01	≅0.05	>0.05
28—33 day	n	9	9	9	9	9	9
	$\bar{x}$	1342	353	153	44.4	257	259.8
	SD	190	72	14.8	4.6	15.9	15.3
	p	>0.05	>0.05	>0.05	<0.05	>0.05	>0.05
34—40 day	n	5	5	5	5	5	5
	$\bar{x}$	1552	193	95.4	39	249.6	264
	SD	148	68.9	19.1	2.6	83.7	46.6

It has been demonstrated by the present study that myocardial infarction is accompanied by changes in the serum-GP levels. The differential character of these modifications clearly emerges from the results.

Studies of GP in a larger numbers of patients and classification of myocardial infarction according to clinical course, severity and possible complications would greatly add to the diagnostic and predictive value of the results. Study of further serum-glycoproteins holds promising, still unexplored possibilities. The ultimate object of these studies should be to establish the GP constellation typical of myocardial infarction.

REFERENCES

1. Antoni, F., T. Szabó, M., Staub, M.: A glycoproteinek biokémiája: Az orvostudomány aktuális problémái. (Ed: Fischer, A.) Medicina, Budapest, (1973).
2. Becker, W., Rapp, W., Schwick, H. G., Storiko, K.: Quantitative determination of plasma proteins by immunoprecipitation. Z. klin. Chem. **6,** 113 (1968).
3. Cleve, H., Strohmeyer, G.: Quantitative Variationen von saurem α_1-glykoprotein, Gc und α_2-macroglobulin mit radialer Immundiffusion. Klin. Wschr. **45,** 1051 (1967).
4. Fehér, J., Jakab, L., Józsa, L.: A serum glycoproteidek szénhydrát componenseinek változása chronicus hepatitisben. Orv. Hetil. **113,** 2880 (1972).
5. Fehér, J., Jakab, L., Réffy, A.: Die Aufteilung und Diagnostik der chronischen Hepatitiden. Z. ärztl. Fortbild., **67,** 113 (1973).
6. Fehér, J., Jakab, L., Szilvási, I., Papp, G.: Die Konzentration einzelner Serumglykoproteine und der Serumimmunglobuline bei der chronischen Hepatitis und Leberzirrhose. Z. ges. inn. Med., **28,** 418 (1973).
7. Hevér, Ö., Eckhardt, S., Tóth, I., Sellei, C.: Haptoglobin vizsgálat értékelése tumoros megbetegedésekben. Orv. Hetil., **113,** 2711 (1972).
8. Jakab, L., Serum Mukopolysaccharid-Untersuchungen bei verschiedenen Erkrankungen. Z. ges. inn. Med. **18,** 1944 (1964).
9. Jakab, L.: A vérben kimutatható glycoproteidekről. In: Az orvostudomány aktuális problémái (Ed.: Fischer, A.). Medicina, Budapest, (1973).
10. Mancini, G., Carbonara, A. O., Heremans, J. E.: Immunochemical quantitation of antigens by single radial immundiffusion. Immunochemistry, **2,** 235 (1965).
11. McMillan, Dc E.: Elevation of glycoprotein fucose in diabetes mellitus. Diabetes **21,** 863 (1972).
12. Miesch, P., Bieth, J., Metais, R.: The α_1-antitrypsin and α_2-macroglobulin content and the protease- inhibiting capacity of normal and pathological sera. Clin. chim. Acta **31,** 231 (1971).
13. Schultze, H. H., Heremans, J. F.: Molecular biology of human proteins. Vol. I., Elsevier, Amsterdam (1966).
14. Snyder, S., Ashwell, G.: Quantitation of specific serum glycoproteins. Clin. chim. Acta, **34,** 449 (1971).
15. Székely, J., Simon, A., Horváth, E.: Serum haptoglobin levels in atherosclerosis. Acta med. Acad. Sci. Hung. **28,** 19 (1971).
16. Tóth, J., Sellei, C., Hevér, Ö., Hindy, I., Eckhardt, S.: Serum coeruloplasmin a vérképzőszervek rosszindulatú megbetegedéseiben. Orv. Hetil. **113,** 2816 (1972.)
17. Winzler, R. J.: Glycoproteins and glycosaminoglycans in plasma and some other body fluids. In: The amino sugars. The chemistry and biology of compounds containing amino sugars. Vol. II. A. Ed.: Balázs, E. A., Jeanloz, R. W., Academic Press, New York (1965).
18. Zawadski, Z. A., Edwards, G. A.: Pseudoparaproteinaemia due to hypertransferrinaemia. Amer. J. clin. Path. **54,**2 80 (1970).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (1—2), pp. 77—83 (1974)

LEUKOTACTIC EFFECT OF HETEROLOGOUS MILK IN RABBIT SKIN

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(Received November 6, 1973)

Fresh human milk and cow's milk of 2.8% fat content exert a marked leukotactic activity on being administered intradermally to rabbits and are thus capable of inducing the preparatory phase of the local Shwartzman reaction. The injection is followed by an inflammatory skin reaction with extensive leukocytic infiltration of the corium.

Endotoxin or 5 to 8 ml milk injected intravenously 24 hours after the preparatory injection elicits a local thrombohaemorrhagic reaction reminiscent in many respects of the local Shwartzman reaction.

Production of the preparatory phase of the reaction is attributed to the powerful leukotactic activity of milk.

The conventional procedure for inducing a local Shwartzman reaction (LSR) in rabbits consists in the intradermal administration of a preparatory dose of endotoxin, followed by a second, provocative, dose of endotoxin administered by the intravenous route.

Earlier, the endotoxin has been prepared by the procedure of BOIVIN and MESROBEANU [1]. Recently, the deproteinized lipopolysaccharide (LPS) derived from Gram-negative bacteria by the method of WESTPHAL et al. [11] has come into use.

We have reported earlier that, besides endotoxin, other macromolecules, such as casein, peptone [7] or even platelets [9] are also suitable for production of the preparatory phase. These agents have the capacity of eliciting a local aggregation of leukocytes in the skin at the site of injection, which is a "sine qua non" for the production of the actual reaction.

In view of the confirmed leukotactic activity of casein in vitro [2] and in vivo [5, 7] and hence, of its being suitable for the production of the preparatory phase of LSR, we have examined human and bovine milk for leukotactic activity in rabbit skin. It has also been attempted to provoke a skin-reaction of the LSR-type by this means.

Material and methods

Ninety rabbits of identical breed and either sex, weighing 2500 ± 500 g were used. The experiments were spread over the four seasons of the year. The animals were kept on standard food supplemented with vegetables. The skin of the back was depilated the day before the experiment.

Grouping of the animals and the treatment schedules have been summed up in Table I.

The milk serving for the studies was obtained from healthy women without evidence of mastitis, 5 to 7 days following delivery. After meticulous breast toilet the milk was collected into sterile containers. The first few ml having been discarded, 0.3 ml of the milk was injected intradermally within 5 to 10 minutes into the skin-areas prepared in the manner described above. At the same time, samples were subjected to bacterial examination.

Five animals were injected in a similar way with commercial pasteurized cow's milk of 2.8% fat content.

In 20 animals provocation of the thrombohaemorrhagic reaction was done by the intravenous injection of 200 μg per rabbit LPS (E. coli 0111 endotoxin, Westphal type, Difco Laboratories, Detroit, Mich).

Thirty rabbits were injected intravenously with 5 to 8 ml human, 10 rabbits with the same amounts of bovine milk 24 hours after intradermal milk injection, of an amount varying with body weight.

The skin lesions thus produced were read 24 hours later by inspection of the inside of the skin.

Specimens were excised from the inflammatory areas of preparation and from the haemorrhagic areas of provocation, fixed in 10% formalin and the sections were stained with haemalaun-eosin and examined under the light-microscope.

Ten rabbits were injected with 8 ml cow's milk into the ear vein on two occasions at 24-hr intervals, with the aim of examining the organs for changes typical of the generalized Shwartzman reaction (GSR).

Results

Results are seen in Table I.

Table I

Leukotactic activity of human bovine milk and provocation of thrombohaemorrhagic skin reaction

Aim of the experiment	Form of treatment	Number of animals	Results
Study of leukotaxis (without provocation)	0.1—0.3 ml human milk intradermally	15	15/15*
	0.1—0.3 ml bovine milk intradermally	5	5/5
Provocation of thrombohaemorrhagic reaction after preparatory phase induced by 0.3 ml human milk	200 μg LPS intravenously	20	16/20
	5—8 ml human milk intravenously	30	19/30
	5—8 ml bovine milk intravenously	10	6/10
Provocation of GSR	2×8 ml bovine milk intravenously	10	0/10

* Numerator: number of animals with positive reaction.
Denominator: total number of animals in the group.

Intradermal injection of 0.1 to 0.3 ml milk into the back skin of rabbits was invariably followed by marked hyperaemia and swelling at the site of injection by the end of 8 to 24 hours. Extension of the inflammatory area seemed to be related to the amount of milk injected. The human or bovine origin of the milk did not affect the intensity of reaction. On the other hand, in the absence of provocation, the inflammatory areas remained free from any haemorrhagic reaction reminiscent of LSR over the next 72 hours.

When 24 hours after the preparatory injection 200 μg LPS or 5 to 8 ml milk were injected into the ear vein, a thrombohaemorrhagic reaction was noted in the areas of inflammation in the course of the subsequent 24 hours. The reaction was reminiscent of a LSR of minor intensity induced by the conventional procedure, i.e. preparation and provocation by means of endotoxin.

It was none the less remarkable that no necrosis had developed, particularly when milk had been employed for provocation.

Microscopic study

Twenty-four hours after administration of human milk, a massive focal leukocytic infiltration of the connective tissue between the epidermis and the skin-muscle layer occurred, without thrombosis or haemorrhages (Fig. 1).

In the central areas occupied by accumulated leukocytes, prevalently by granulocytes, there were capillaries distended with leukocytes. Around the dilated capillaries clusters of migrating leukocytes appeared (Fig. 2).

Under higher power leukocyte migration through the capillary wall was clearly seen (Fig. 3).

Twenty-four hours after milk injection, a thrombohaemorrhagic skin reaction has formed at the site of the previous intradermal preparatory in-

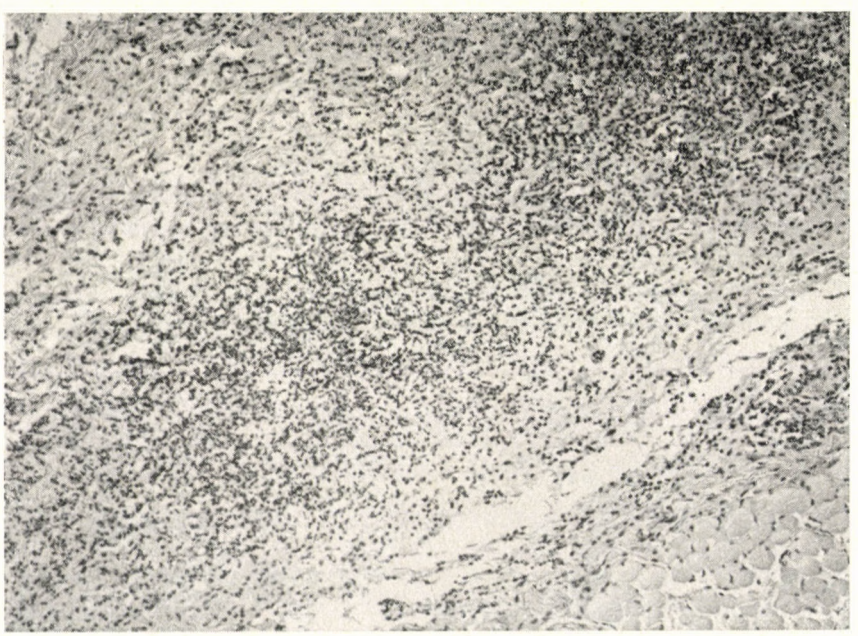

Fig. 1. Rabbit skin 24 hours after intradermal injection of 0.3 ml human milk. Cross-section of a skin muscle in the lower quadrant. In the corium, focal leukocytic infiltration of indistinct outlines. Haemalaun-eosin stain, ×100

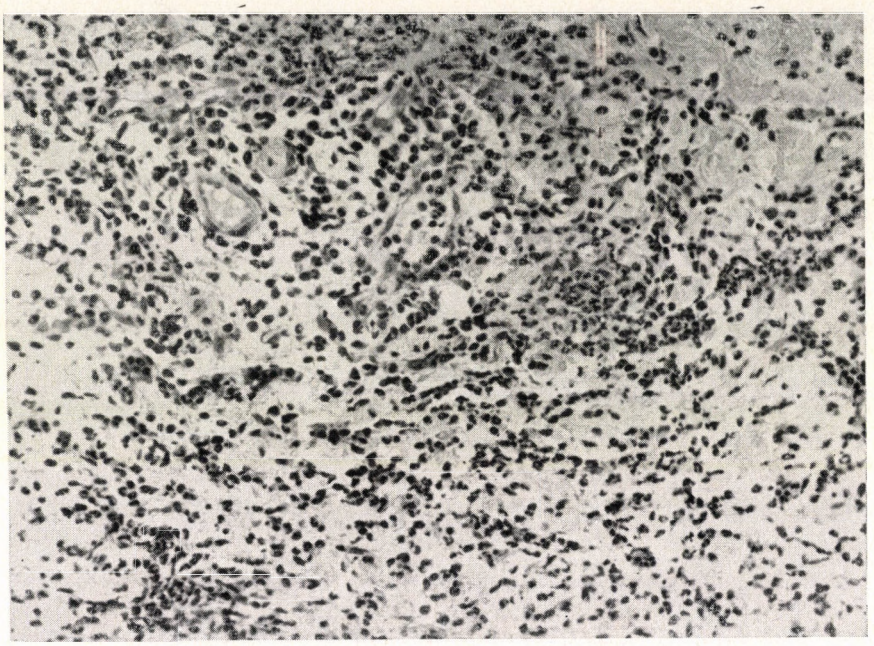

Fig. 2. Rabbit skin 24 hours after administration of human milk. In the central area of the leukocytic accumulation in the corium there are distended capillaries with leukocytes aligned in the lumen along the endothelium. Haemalaun-eosin stain, ×250

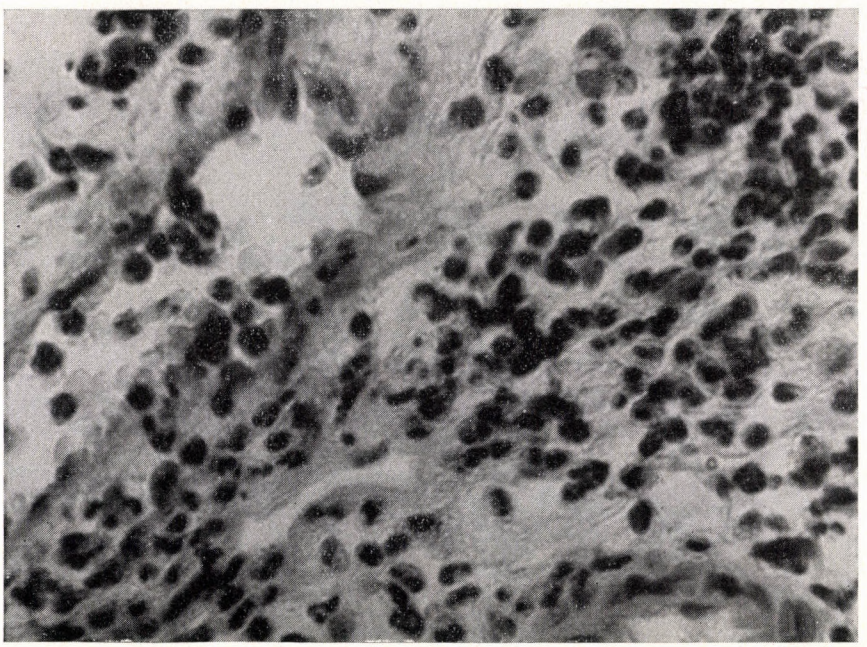

Fig. 3. Leukotaxis induced with human milk in rabbit skin. Invasion of the capillary endothelium by migrating leukocytes. Haemalaun-eosin stain, ×400

jection in approximately two-thirds of the animals. In the distended capillaries clots and around them numerous extravasal red blood cells were seen (Fig. 4). Rarely, crust formation occurred, particularly in endotoxin-treated animals.

The LSR required 5 to 8 ml milk for its provocation in rabbits. Smaller doses, e.g. 1 to 2 ml, produced occasional reactions only, while after 10 to 20 ml, the majority of the animals died soon after the injection.

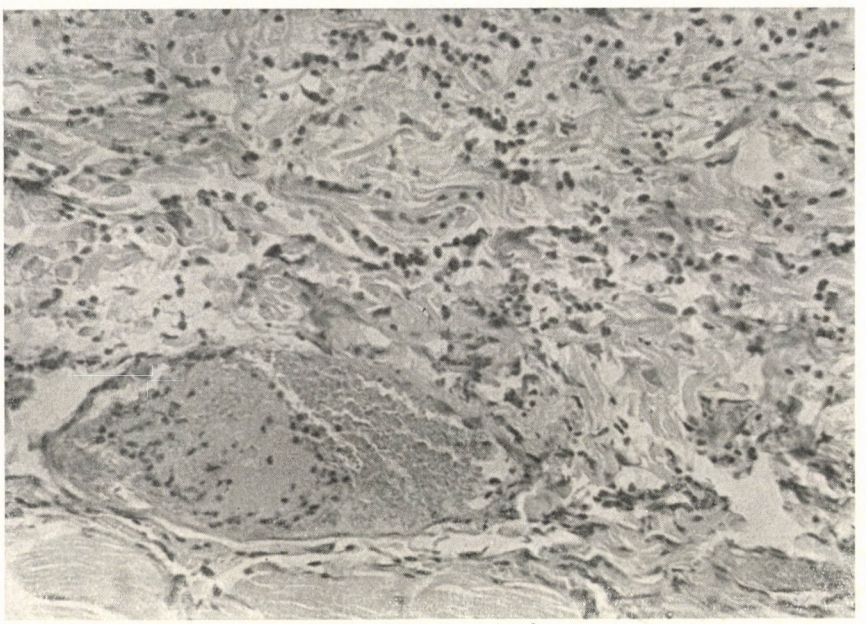

Fig. 4. Thrombohaemorrhagic reaction in rabbit skin 24 hours after intravenous administration of human milk. Thrombus in the lumen of a capillary. Haemalaun-eosin stain, ×250

Administration of 5 to 8 ml milk was followed by prostration of the animals with a rise in rectal temperature up to 40°C. Recovery took 8 to 12 hours.

Production of the reaction reminiscent of LSR was successful all the year round, regardless of the season. The intensity of the reactions culminated in the summer and autumn months. Female, and especially pregnant, rabbits displayed an enhanced responsiveness.

Intensity of the thrombohaemorrhagic skin reaction was not affected by the human or bovine origin of the milk. After repeated administration of 8 ml bovine milk no systemic abnormalities typical of GSR were noted in any of the animals.

No pathogens were demonstrable in any of the human milk samples, the bacterial count was about less than 10^4 per ml. In a few cases, achromogenous bacteria were found.

Discussion

On the evidence of the present study, fresh (5 to 10- minutes- old) human milk exerts a powerful leucotactic effect on rabbit skin. This activity resulting in leukocyte migration is unrelated to the presence of pathogenic bacteria. The presence of endotoxin in any significant amounts may also be ruled out with reasonable certainty, since human milk failed to provoke a GSR, even in repeated doses. The leukotactic activity of milk may be ascribed instead to its natural constituents. On the other hand, the bacterial and possible endotoxin content of bovine milk may be directly involved in the production of leukotaxis.

In view of the powerful leukotactic activity of human milk there might be some reason to connect the appearance of mastitis in the post-partum period with the primary phlogogenic properties of human milk. However, before drawing this conclusion, it should be studied whether the leukotactic effect of human milk was valid for human tissues.

Since the intradermal injection of human or bovine milk results in a local accumulation of leukocytes, subsequent administration of endotoxin is suitable for the provocation of a thrombohaemorrhagic skin reaction.

While in our earlier studies, intravenous casein, peptone or platelet-rich plasma failed to induce a thrombohaemorrhagic reaction, on the evidence of the present study, milk, whether of human or bovine origin, lends itself well to preparation as well as to provocation of an LSR.

Endotoxin is well-known to play a decisive part in the production of the preparatory phase as a result of its leukotactic activity. In the provocative phase, where certain factors affecting microcirculation and blood clotting also play a part, endotoxin is also closely involved by releasing lysosomal enzymes from the leukocytes [3, 6, 8].

A case has been reported recently by WALLACE et al. [10] in which injection of 140 ml of pasteurized cow's milk induced a severe reaction marked by high temperatures, circulatory disturbances, and petechiae on the chest developed immediately followed by a defibrination syndrome. On culturing, the milk sample proved non-pathogenic, although it contained two unidentified Gram-negative strains. The authors considered the milk itself to have been the responsible factor and attributed certain features of the syndrome to fat embolism and to disseminated intravascular coagulation.

In view of this case, provocation of a haemorrhagic skin reaction in laboratory animals by intravenous human or bovine milk might well be attributed, in accordance with the high mortality induced by major doses, to fat embolism, which is liable to produce haemodynamic changes essential to the production of the reaction, parallel with an enhancement of the clotting mechanisms.

Finally, it might be an advantage that milk can be used for the production of a thrombohaemorrhagic skin reaction similar in many respects and lending itself to the experimental study of the LSR.

Acknowledgements

We are greatly indebted to Dr. M. Boján for microbiological studies.

REFERENCES

1. Boivin, A., Mesrobeanu, L.: Rev. Immunol. **1,** 553 (1935).
2. Keller, H., Sorkin, E.: Experientia (Basel) **24,** 611 (1968).
3. Kovács, T.: Endotoxin susceptibility and endotoxin hypersensitivity. Stud. Med. Szeged 1967.
4. Stetson, C., Good, R.: J. exp. Med., **93,** 49 (1951).
5. Szilágyi, T., Csernyánszky, H., Csákó, Gy., Benkő, K.: Experientia (Basel) **27,** 1469 (1971).
6. Szilágyi, T., Tóth, S., Muszbek, L., Lévai, G., Laczkó, J.: Acta microbiol. Acad. Sci. Hung. **15,** 331 (1968).
7. Tóth, S., Szilágyi, T., Went, M., Lévai, G.: Acta phys. Acad. Sci. Hung., **42,** 163 (1972).
8. Tóth, S., Szilágyi, T., Károlyi, G., Lévai, G.: Acta med. Acad. Sci. Hung. **29,** 271 (1972).
9. Tóth, S., Muszbek, L., Szilágyi, T., Lévai, G., Laczkó, J.: Experientia (Basel) **25,** 1085 (1969).
10. Wallace, J., Payne, R., Mack, A.: Lancet **1,** 1264 (1972).
11. Westphal, O., Lüderitz, O., Bister, F.: Z. Naturforsch. **7b,** 148 (1952).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (1—2), pp. 85—90 (1974)

ÜBER VERÄNDERUNGEN DER VERESTERTEN FETTSÄUREN — TRIGLYCERIDE UND CHOLESTEROLESTER — IM BLUTPLASMA BEI EXPERIMENTELLER HYPERTONIE DER RATTE

Von

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(Eingegangen am 12. November 1973)

Es wurden die veresterten Fettsäuren — Triglyceride und Cholesterolester — im Blutplasma in einem Hypertoniemodell an der Ratte untersucht. Es wurde ein signifikanter Anstieg der ungesättigten Reihe der veresterten Fettsäuren (TG) verzeichnet, parallel mit entsprechenden Veränderungen im Spektrum der freien, nicht veresterten Fettsäuren im Blut. Bei hypertoner Belastung nimmt die Palmitinsäure den vorherrschenden Platz der gesättigten Reihe, die Ölsäure den der ungesättigten Reihe ein. Diametral gestalten sich die Veränderungen der veresterten Fettsäuren (Cholesterolester) bei hypertensivem Zustand. Hier sind die gesättigten Fettsäuren vermindert und die ungesättigten entsprechend erhöht. Die biologisch wichtigen essentiellen Fettsäuren, Linol- und Arachidonsäure, sind angestiegen.

Es wurden einige Aspekte des Fettstoffwechsels in Verbindung mit der erhöhten energetischen und funktionellen Bedürfnissen bei Hypertonie diskutiert. Die Veränderungen der veresterten Fettsäuren sind als ein adaptiver Mechanismus zu betrachten.

Die endogenen Triglyceride (TG) werden von der Leber produziert und in das Blutplasma als Lipoproteine mit sehr niedriger Dichte (very low density lipoproteins — VLDL) sekretiert, während die Chylomikronen-TG, die exogenen Ursprungs sind, vom Intestinum über den Ductus thoracicus in das Plasma gelangen. Die Konzentration der TG im Blut ist abhängig von der Bilanz ihres In- und Output, bzw. ihre Umsetzung und Hydrolysierung zu freien Fettsäuren (FFS), welche weiterhin aktiv am gesamten Metabolismus teilnehmen. Offensichtlich haben Chylomikronen-TG Bedeutung als energetische Fettreserve, während die endogenen TG nach ihrer Lipolyse im Blut sofort am oxydativen Stoffwechsel teilnehmen können. Eine positive Korrelation zwischen der Intensität des FFS-Umsatzes im Plasma und dem Umfang der Entstehung der FFS aus TG wurde festgestellt [1]. Sowohl bei physischer Belastung [2] als auch bei Hypertonie [3] wurde eine Senkung des Blutspiegels der TG gefunden, welche mit einer erhöhten Utilisation bei einem alternativen Verbrauch der Substrate in der Oxydation interpretiert werden kann.

Veresterte Fettsäuren im Blutplasma in Form von Cholesterolester werden vorwiegend in der Leber sekretiert und katabolisiert [4, 8]. Man nimmt

an, daß die physiologische Rolle der Cholesterolester mit dem Transport von Lipiden verbunden ist [5] und daß diese keine Bedeutung als Energiequelle besitzen. Ihre Konzentration im Blut steht wahrscheinlich im Zusammenhang mit den funktionellen Bedürfnissen nach Baumaterial für Lipoproteine und für die Steroidgenese. Beim Mechanismus der Freisetzung von Fettsäuren aus den Lipoproteinen und ihrer nachfolgenden Aufnahme in die Zellen spielen zwei Enzyme eine gewichtige Rolle: Lipoproteid-Lipase (clearing factor — LPL) und Lecithin–Cholesterol–Acyl-Transferase (LCAT). Während durch die LPL die Fettsäuren aus den TG freigesetzt werden, greift die LCAT vor allem die Lecithinhülle der Lipoproteine an. Es kommt somit zu einer synchronen Verkleinerung der Lipoproteine und zu einer Freisetzung ihres Inhaltes — FFS, Glycerole, Cholesterol u. a. Dieser Mechanismus läuft in zwei Richtungen mit alternierendem Charakter ab und ordnet sich den klassischen Gesetzen der Kinetik der fermentativen Katalyse unter.

Die Regulation des Metabolismus der veresterten Fettsäuren — TG und Cholesterolester — bei intakten Tieren, und zwar ihre Synthese, Absorption und Destruktion, wird als Komplex verzahnter Mechanismen betrachtet. Die hormonsensitiven Enzyme vermitteln über drei Hauptwege, über das sympathische Nervensystem mit Catecholaminen, über den Nervus vagus mit Insulin und über die Proteinsynthese mit Nebennierenrindenhormonen und dem Wachstumshormon, den Einfluß der Faktoren des inneren und äußeren Milieus.

Ausgehend von der Tatsache, daß im Hungerzustand bei der Zusammensetzung der TG im Blutplasma die endogenen TG überwiegen, stellten wir uns die Aufgabe, die Veränderungen des Musters der veresterten Fettsäuren — TG und Cholesterolester — bei hypertoner Belastung am Tier zu untersuchen und mit normotonen Bedingungen zu vergleichen.

Material und Methoden

Die Untersuchungen des Musters der veresterten Fettsäuren — TG und Cholesterolester — führten wir an 50 Albinoratten durch; die gleiche Anzahl Ratten diente als Kontrollgruppe (Stamm Wistar, beiderlei Geschlechts, ca. 220 g, 9 Monate alt, 12stdg. Nahrungskarenz). Ein experimentelles Modell der neurogen-interorezeptiven Hypertonie erzeugten wir durch beiderseitiges Entfernen der Barorezeptoren des Sinus caroticus zusammen mit den Fasern des aortendepressorischen Nerven [6] und entnahmen nach der Stabilisierung des erhöhten Blutdruckes (Mittelwerte 170/115 Torr nach den Ausgangswerten 105/65 Torr) das Blut für die Untersuchungen. Die Blutdruckmessung erfolgte mit Hilfe eines Kondensatormikrophons nach Boucke—Brecht am Schwanz des nicht narkotisierten Tieres. Für die Dokumentation wurde die Pulskurve durch einen Elektrokardiographen photographisch aufgezeichnet. Das Blut wurde durch Herzpunktion entnommen. Die Extraktion der Lipide erfolgte mit Chloroform-Methanol; die Lipidfraktionen trennten wir durch Dünnschichtchromatographie mit Kieselgel G/Merck, flüssige Phase: Hexan–Diäthyläther-Essigsäure, 85 : 15 : 2,5 V/V/V. Die Umesterung nahmen wir mit 0,25 nNa-Methylat vor [7].

Die Untersuchungen des Musters der veresterten Fettsäuren wurde mittels eines Gaschromatographen (Gasofract TP 500, Fa. Dr. Virus KG, Bonn) mit Flammenionisationsdetektor durchgeführt. (Die gaschromatographischen Bedingungen waren folgende: Säule: 300 × 0,4 cm Kupferrohr; Säulenfüllung: 7,5% Diäthylenglycolsuccinat auf Gaschrom Q, 100—120

mesh; Temperaturen: Injektionspunkt 260°C, Säule 185°C, Detektor 265°C; Trägergas: Stickstoff, 2,8 l/h Durchfluß; Empfindlichkeit: $0{,}5 \times 10^{-9} - 1{,}0 \times 10^{-9}$ A; Papiervorschub: 600 mm/h.) Die Ergebnisse wurden statistisch ausgewertet unter Anwendung des *t*-Testes und der Korrelationsprüfung.

Ergebnisse

I. Die Veränderungen im Muster der veresterten Fettsäuren — TG — bei Hypertonie zeigen wir in Tabelle I. Sie sind ausgeprägt nach folgenden Richtungen:

Tabelle I

C—Zahl	Normotontiere %	Hypertontiere %	
I. Gesättigte veresterte Fettsäuren — Triglyceride			
C_{12}	1,6 ± 0,2	2,4 ± 0,8	
C_{14}	1,4 ± 0,2	1,6 ± 0,2	
C_{16}	42,2 ± 3,4	45,9 ± 3,7	($p < 0{,}01$)
C_{18}	2,6 ± 0,3	4,2 ± 0,5	($p < 0{,}05$)
Σ	47,8	54,1	
II. Ungesättigte veresterte Fettsäuren — Triglyceride			
$C_{16:1}$	10,3 ± 1,0	8,5 ± 0,8	($p < 0{,}05$)
$C_{18:1}$	32,6 ± 2,5	24,7 ± 2,1	($p < 0{,}01$)
$C_{18:2}$	7,6 ± 0,7	9,6 ± 0,9	($p < 0{,}05$)
$C_{18:3}$	0,7	1,0	
$C_{20:4}$	1,0	2,1 ± 0,3	
Σ	52,8	45,9	

1. Erhöhung der gesättigten Fettsäuren; alle untersuchten Vertreter zeigen erhöhte Werte, vorwiegend die Palmitinsäure,

2. Verminderung der Reihe der ungesättigten Fettsäuren, insbseondere der Ölsäure,

3. polyene Fettsäuren (essentielle Fettsäuren) zeigen eine gewisse Vermehrung.

II. Die Veränderungen des Musters der veresterten Fettsäuren — Cholesterolester — bei experimenteller Hypertonie demonstriert Tabelle II. Sie verlaufen zum größten Teil diametral zu den obigen Resultaten, und zwar:

1. Die ganze Reihe der gesättigten Fettsäuren zeigt verminderte Werte, besonders die Palmitinsäure.

2. Die ungesättigten Fettsäuren sind vermehrt; diese Veränderungen aber konstatierten wir vor allem bei den polyenen Fettsäuren, so bei der Linol- und Arachidonsäure. Die Vermehrung weist Parallelität zu den korrespondierenden Fettsäuren der TG auf, ist hier jedoch signifikant.

Tabelle II

C–Zahl	Normotontiere %	Hypertontiere %	
I. Gesättigte veresterte Fettsäuren — Cholesterolester			
C_{12}	1,2±0,3	1,5±0,4	
C_{14}	4,4±0,6	3,4±0,5	
C_{16}	28,1±2,9	24,6±2,6	($p < 0,05$)
C_{18}	1,4±0,3	1,2±0,2	
C_{20}	1,0±0,2	0,2	
Σ	36,1	30,9	
II. Ungesättigte veresterte Fettsäuren — Cholesterolester			
$C_{16:1}$	16,1±1,5	14,9±1,3	
$C_{18:1}$	22,6±2,8	19,0±1,8	($p < 0,05$)
$C_{18:2}$	9,8±1,4	16,5±1,7	($p < 0,01$)
$C_{18:3}$	2,6±0,3	3,5±0,3	($p < 0,05$)
$C_{20:4}$	12,8±1,2	15,2±1,8	($p < 0,01$)
Σ	63,9	69,1	

Diskussion

Bei hypertoner Belastung des Organismus, welche ein Beispiel für einen energieerfordernden Zustand darstellt, wird ein breiterer Umfang der veresterten Fettsäuren — TG — in die für den energetischen Metabolismus benötigten freien Fettsäuren umgesetzt. Auch ein vermehrter Umfang von veresterten Fettsäuren — TG — wird von der Leber produziert und in das Blut sekretiert. Bei der bedeutenden Erhöhung der Umsatzrate der TG im Plasma wird ihre Utilisation überstiegen. Eine positive Korrelation besteht zwischen dem Logarithmus der Werte des TG-Umsatzes im Blutplasma und der Aktivität der LPL (Klärungsfaktor-Lipase) [8]. Die Quelle dieses Enzyms ist die Endothelialmembran des Gefäßsystems und besonders die Vaskularität der Leber [9]. Bei Hypertonie wurden für die Aktivität dieser Lipase größere Werte gefunden [10], welche auf erhöhte energetische Forderungen zurückzuführen ist. Aus der Verminderung der Aktivität dieses Faktors resultieren eine Vergrößerung des TG-Niveaus und sklerotische Prozesse in der Gefäßwand mit Veränderungen im Metabolismus der Mucopolysacharide. Die Hyperglyceridämie bei Diabetes steht im Zusammenhang mit einer verminderten Aktivität der

LPL bei Hypoinsulinämie und einer vergrößerten Konversion der Kohlenhydrate in der glukoseabhängigen Lipogenesis.

Die Veränderungen des Musters der veresterten Fettsäuren — TG — im Blutplasma verlaufen in hohem Grad parallel mit entsprechenden Veränderungen der FFS im Plasma bei experimenteller Hypertonie mit der relativen Vergrößerung des Teiles der gesättigten Fettsäuren und mit der relativen Verminderung des Anteils der ungesättigten Fettsäuren [11]. Während bei einem FFS-Verbrauch sich die essentiellen Fettsäuren, so die Linol- und Arachidonsäure, gefolgt von der Ölsäure, beträchtlich vermindern, wechselt diese Ordnung diametral bei dem Muster der veresterten Fettsäuren. In manchen Untersuchungen wurde bei Anwendung von Noradrenalin oder Adrenalin die Synthese von Cholesterolester und endogenen TG erhöht gefunden [12], ferner daß bei einer Belastung des Organismus, z. B. bei physischer Arbeit, der Umsatz von Lipoproteinen mit Cholesterolester erhöht ist [13]. Die Aktivität der Cholesterolesterase in der Leber trägt zu einer vermehrten Veresterung von ungesättigten als von gesättigten Fettsäuren bei. Bei hypertoner Belastung hat die Leber gesteigerte funktionelle Aufgaben, und die Sekretion von LCAT kann sich erhöhen. Unser Befund weist auf einen vermehrten Transport von ungesättigten veresterten Fettsäuren hin. Die LCAT zeigt bei Belastungszustand des Organismus breitere Aktivität, und zwar betrifft dies in erster Linie die Veresterung von Linol- und Arachidonsäure. Eine ähnliche Erscheinung tritt bei Hungerzuständen auf [14].

Der Umsatz der FFS in der Blutzirkulation verhält sich proportional zu ihrer Konzentration im Plasma [15]. Bei normalen Bedingungen besteht ein stabiles Verhältnis zwischen dem Zufluß und der Utilisation der FFS. Jede einzelne FFS wird von der Leber in unterschiedlichem Grad aufgenommen und auch unterschiedlich metabolisiert [16, 17]. Der Vorgang, durch den die Leber die FFS diskriminiert, ist unbekannt. Das Linoleat wird von der Leber für die Inkorporation in die Cholesterolester und die Phospholipide bevorzugt [18].

Eine hypertone Belastung des Organismus erzeugt Veränderungen des Musters der veresterten Fettsäuren — TG und Cholesterolester, — welche bestimmte physiologische Funktionen im Metabolismus der Hypertonie besitzen. Einerseits dienen die TG als Reserve für die FFS im oxydativen Metabolismus, andererseits dienen TG und besonders Cholesterolester außer als Baumaterial für die Lipoproteine auch den erweiterten Bedürfnissen nach polyenen Fettsäuren. Die Verminderung der polyenen Fettsäuren — Cholesterolester und TG — wie auch die Einschränkung der Hydrolysierung von TG ist ein Merkmal für eine verringerte funktionelle Adaptation der physiologischen Systeme des Organismus, welche zu Verteidigungsreaktionen führt. Durch diese metabolischen Alterationen kann der Organismus der Fluktuation der Bedingungen des inneren und äußeren Milieus entsprechen.

LITERATUR

1. MILLER, H. I.: Plasma-free fatty-acid appearance in plasma triglycerides. Metabolism **16,** 1096 (1967).
2. CARLSON, L. A. and FRÖBERG, S. O.: Blood lipid and glucose levels during a ten-day-period of low-caloric intake and exercise in man. Metabolism **16,** 624 (1967).
3. MICHAILOV, M. L., NITSCHKOFF, HOLLSTEIN, E. und BAUMANN, R.: Über Veränderungen des Spektrums der veresterten Fettsäuren (Triglyceride) im Blutplasma bei experimenteller nephrogener Hypertonie an der Ratte. Dtsch. Gesundh.-Wes. **26,** 763 (1971).
4. DEYKIN, D. and GOODMAN, DE W. S.: The hydrolysis of long-chain fatty acid ester of cholesterol with rat liver enzymes. J. biol. Chem. **237,** 3649 (1962).
5. NESTEL, P. J.: Turnover of plasma esterified: Influence of dietary fat and carbohydrate and relation to plasma lipids and body weight. Clin. Sci. **38,** 593 (1970).
6. KRIEGER, E. M.: Neurogenic hypertension in the rat. Circulat. Res. **15,** 511 (1964).
7. OETTE, K., DOSS, M. and WINTERFELD, M.: Umesterung und Veresterung von lang- und kurzkettigen Fettsäuren in Kapillaren für die Gaschromatographie. Z. klin. Chem. **8,** 525 (1970).
8. SAILER, S., SANDHOFER, F. und BRAUNSTEINER, H.: Steuerung der endogenen Lipoproteid-Lipase-Aktivität im Plasma bei Normalpersonen und Patienten mit essentieller Hyperlipämie. Dtsch. med. Wschr. **90,** 865 (1965).
9. NAITO, Y. C. and FELTS, J. M.: Influence of heparin on the removal of serum lipoprotein lipase by the perfused liver of the rat. J. Lipid Res. **11,** 48 (1970).
10. MALLOV, S.: Aortic lipoprotein lipase activity in relation to species, age, sex and blood pressure. Circulat Res. **14,** 357 (1964).
11. MICHAILOV, M. L.: Untersuchungen der nicht veresterten Fettsäuren bei experimenteller Hypertonie an der Ratte. Acta med. Acad. Sci. Hung. **28,** 267 (1971).
12. BORTZ, W. M.: Noradrenaline-induced increase in hepatic cholesterol synthesis and its blockade by puromycin. Biochim. biophys. Acta (Amst.) **152,** 619 (1968).
13. MALINOW, M. R., PERLEY, A. and MCLAUGHLIN, P.: Muscular exercise and cholesterol degradation: Mechanismus involved. J. appl. Physiol. **27,** 662 (1969).
14. SWELL, L. and LAW, M. D.: Influence of fasting on the formation of cholesterol arachidonate by the serum cholesterol esterifying enzyme. Proc. Soc. exp. Biol. (N. Y.) **129,** 363 (1968).
15. ISSEKUTZ, B., BORTZ, W. M., MILLER, H. I. and PAUL, P.: Turnover rate of plasma FFA in human and in dogs. Metabolism **16,** 1001 (1967).
16. NESTEL, P. J., BEZMAN, A. and HAVEL, R. J.: Metabolism of palmitate and linoleate in intact dogs. Amer. J. Physiol. **203,** 914 (1962).
17. MILLER, H. I., GOLD, M. and SPITZER, J. J.: Removal and mobilisation of individual free fatty acids in dogs. Amer. J. Physiol. **202,** 370 (1962).
18. ORTH, R. D., FINE, M. B. and WILLIAMS, R. H.: Incorporation of infused palmitic-1-C^{14} and linoleic-1-C^{14} into plasma lipid fractions. Proc. Soc. exp. Biol. (N. Y.) **106,** 339 (1961).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (1—2), pp. 91—94 (1974)

PROTEOLYTIC ACTIVITY OF HUMAN GASTRIC JUICE

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(Received November 13, 1973)

The proteolytic activity of human gastric juices was investigated using bovine albumin and casein as substrates. It was found that casein was better digested by 73% and albumin by 27% of the tested subjects. The finding stresses the necessity of estimating proteolytic activity in human gastric juice by using both substrates.

For the estimation of proteolytic activity in human gastric juice by the method of BITSCH [1], serum protein is used as a substrate, as the composition of food proteins usually does not correspond to the composition of serum protein. This leads to an erroneous evaluation of the proteolytic activity of gastric juice, since we do not know whether the individual isoenzymes of pepsin have the same substrate specificity and kinetic properties. It is not known to what degree the inhibition of gastric secretion influences the activity of a particular isoenzyme.

The aim of the present investigation was to determine the proteolytic activity of gastric juices, using two substrates i.e. casein and albumin. Estimation of proteolytic activity was performed in specimens obtained at basal secretion as well as during inhibition and stimulation of gastric secretion.

Material and methods

Sample preparation

From 48 healthy normochlorhydric persons two specimens of gastric juice were collected, each for one hour, according to the method of KAY [2]: the first basal (BAO secretion) and the second after administration of a stimulating or inhibitor substance. The subjects were divided into three groups of 16 persons each. After estimation of the basal secretion, to the subjects in the first group histamine hydrochloride (p.024 mg/kg body weight), to those in the second group, crystalline insulin (0.2 unit/kg body weight) and in the third group, atropine sulphate (0.001 mg/kg body weight) was administered. Each sample was filtered through 16 layers of wet gauze and titrated with 0.1 N sodium hydroxide using phenol red as indicator.

Estimation of proteolytic activity

Proteolytic activity was estimated by the method of BITSCH [1], using casein (BDH) or bovine albumin (SEVAC) as a substrate. The gastric juice was diluted immediately before analysis with an equal volume of hydrochloric acid solution, pH 1.9. The calibration curve was obtained for both substrates separately, using increasing amounts of triple crystallized pepsin

(Koch-Light). The casein substrate was prepared as follows: 5.3 g casein was dissolved in 100 ml of hydrochloric acid solution of pH 1.6 under magnetic stirring at room temperature 1—2 hours. The slightly turbid solution contained 4.4—4.8 g/100 ml of protein, as determined by the method of LOWRY et al. [3]. The albumin substrate was prepared as follows: 5.3 g bovine albumin was dissolved in 100 ml of hydrochloric acid solution at pH 1.6 on a magnetic stirrer at room temperature. The bovine albumin concentration was 4.4—4.8 as determined by the method of LOWRY et al. [3] .Until use each substrate was kept in the deep freeze. The gastric juice was tested in duplicate with a blank, as described by BITSCH [1].

Results

Results are summarized in Table I and Fig. 1. Estimation of proteolytic activity in BAO secretion showed that of the 48 subjects, casein was digested

Table I

Hydrochloric acid secretion and proteolytic activity

	Stimulus	No. of subjects	Group I n = 35 $\bar{x}$ SD	No. of subjects	Group II n = 13 $\bar{x}$ SD	No. of subjects	Statistic significance of difference between group I and II
Proteolytic activity to casein as substrate (expressed in mg pepsin per 1 l of gastric juice)	atropine	16	308±128	11	135± 54	5	
	bao	48	552±186	35	266± 92	13	$p \leq 0.02$
	histamine	16	1231±362	12	550±201	4	
	insulin	16	1675±420	12	800±278	4	$p \leq 0.001$*
Proteolytic activity to albumin as substrate (expressed in mg pepsin per 1 l of gastric juice)	atropine	16	200± 76	11	355±121	5	
	bao	48	403±162	35	583±202	13	$p \leq 0.02$
	histamine	16	926±283	12	1366±320	4	
	insulin	16	1180±262	12	1675±302	4	$p \leq 0.001$*
Hydrochloric acid output mEq/hour	atropine	16	0.49±2.0	11	0.96±0.3	5	
	bao	48	2.9±1.2	35	5.6±1.6	13	$p \leq 0.001$
	histamine	16	13.6±4.2	12	22.6±6.3	4	
	insulin	16	6.2±2.0	12	11.7±3.6	4	$p \leq 0.001$*

* Calculated for histamine and insulin together.

better by 35 subjects and albumin was digested better by 13 subjects. After administration of atropine, histamine or insulin, each subject retained the BAO digestion pattern. In each of the two groups treated with histamine and insulin, respectively, there were 12 subjects who digested casein better and 4 subjects who digested albumin better. The differences between the two groups in secretion before and after administration of histamine and insulin were significant statistically ($p \leq 0.02$ to $p \leq 0.001$).

Table I and Fig. 1 show the values for proteolytic activity and hydrochloric acid output of the tested subjects. There was a positive correlation between hydrochloric acid output and proteolytic activity against albumin as the substrate and no correlation with casein.

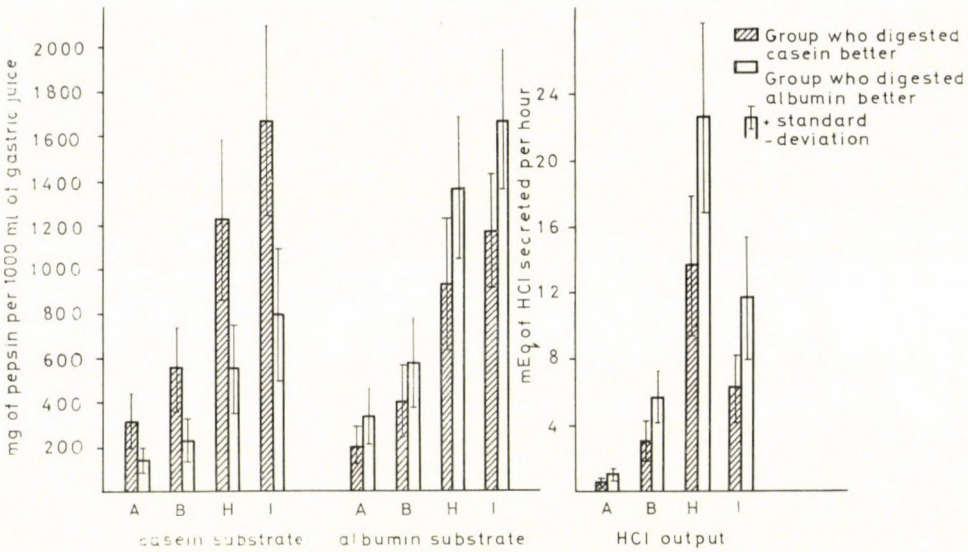

Fig. 1. Proteolytic activity with casein and albumin as substrates, and hydrochloric acid output. Gastric juice after administration of atropine (A), histamine (H), and insulin (I) respectively. (B) — basic secretion

Discussion

In a group of healthy normochlorhydric subjects, 73% were found to digest casein better and 27% to digest albumin better. Other serum proteins were digested similarly as serum albumin. The ability to digest casein or albumin better is an individual characteristic unrelated to the stimulus applied. For example, a subject examined three times at 1—2 monthly intervals, digested only casein in BAO secretion as well as after stimulation by histamine and insulin. The better digestion of casein or albumin is probably related to differences in the composition of pepsin isoenzymes [4], possibly determined by the genetic factors. Hypoglycaemia caused a copious excretion of pepsin, whereas histamine enhanced hydrochloric acid excretion.

The results indicate that casein, in spite of some technical difficulties, is a better substrate than albumin for the testing of proteolytic activity, but the simultaneous use of both substrates is recommended.

REFERENCES

1. BITSCH, V.: A modification of the Hundt method for estimating peptic activity in gastric juice. Scand. J. Clin. lab. Invest. **18,** 357—358 (1966).
2. KAY, A. W.: Effect of a large dose of histamine on gastric secretion of HCl. Brit. med. J. **2,** 77—83 (1953).
3. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., RANDALL, R. J.: Protein measurement with the Folin phenol reagent, J. biol. Chem. **193,** 265—275 (1951).
4. SAMLOFF, I. M., TOWNES, P. L.: Electrophoretic heterogeneity and relationship of pepsinogens in human urine, serum and gastric mucosa. Gastroenterology **4,** 462—469 (1970).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (1—2), pp. 95—98 (1974)

PLASMA CELL MYOSITIS IN RHEUMATOID ARTHRITIS

By

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(Received November 26, 1973)

A 46-year-old man with a three-month history of definitive rheumatoid arthritis has undergone synovectomy of the right wrist. Muscle biopsies taken at this time showed large plasma cell infiltrates. The immunoglobulins were normal. Ten months later, biopsy of the same muscles showed no evidence of the plasma cell infiltration. It is concluded that the plasma cell infiltrates in the muscles might be an early manifestation of rheumatoid disease.

Plasma cell myositis in rheumatoid arthritis

The striated muscle in rheumatoid arthritis shows a number of abnormalities which can be recognized both clinically and histologically (CURTIS and POLLARD [2], SOKOLOFF et al. [7] STEINER and CHASON [8], WITTENBORG [9]). The changes involve the contractile elements, the sarcoplasm, as well as the interstitium. One of the common histological findings is the presence of nodular myositis, which manifests itself with perivascular clusters of lymphocytes and lymphorrhages. These findings are considered typical, although not pathogenomonic of rheumatoid arthritis (GARDNER [4], GIBSON et al. [5]). Infiltration of the muscle interstitium by histiocytes, and proliferation of fibroblasts are also common findings (BENEKE [1]).

No reports on extensive, pure plasma- cell infiltration of striated muscles in rheumatoid arthritis were found in the literature. A case exhibiting these changes is described here. No other cases displaying such features were found among 70 patients with rheumatoid arthritis.

Report of a case

The patient, a 46-year-old man, had been suffering from pain affecting both shoulders wrists and the right hip for 3 months. Clinical examination revealed a picture of definitive rheumatoid arthritis, with swelling and tenderness of the right wrist, the metacarpophalangeal and proximal interphalangeal joints of both hands, and tenderness of both shoulders.

No radiological abnormalities were detected. Laboratory findings revealed a raised ESR (68 mm/hr Westergren). The haemoglobin amounted to 12.6 g/100 ml.

The Rose-Waaler test was negative. All the biochemical investigations, including serum electrophoresis and immunelectrophoresis, were normal. The patient was treated with phenyl-

butazone, and by injections of hydrocortisone into the shoulder joints. Synovectomy of the right extensor digitorum muscle was performed. Postoperative recovery was uneventful and there was a considerable improvement in the patient's condition.

Ten months after the original operation, a muscle biopsy was made from the extensor digitorum and extensor carpi ulnaris muscles. The Rose—Waaler test was still negative, and the biochemical findings were normal.

The patient remains well and is in remission 11 months after the synovectomy.

Pathology

The synovectomy specimen consisted of a swollen, oedematous tendon sheath and a few small fragments of striated muscle. Microscopically the tendon sheath was congested and oedematous, containing many blood vessels with marked perivascular cuffing by lymphocytes and plasma cells. There were foci of necrosis, and the surface was covered by fibrin deposits. The striated muscle showed atrophic changes in some muscle fibres, which contained prominent sarcolemmal nuclei. The sarcoplasm showed loss of the cross striation in some areas. There were large collections of plasma cells in the endomysium and perimysium, and they were infiltrating the muscle fibres (Figs 1, 2). The plasma cell infiltration was extensive, and was equally marked in the extensor digitorum communis muscle, and the extensor carpi ulnaris, the muscle which was biopsied.

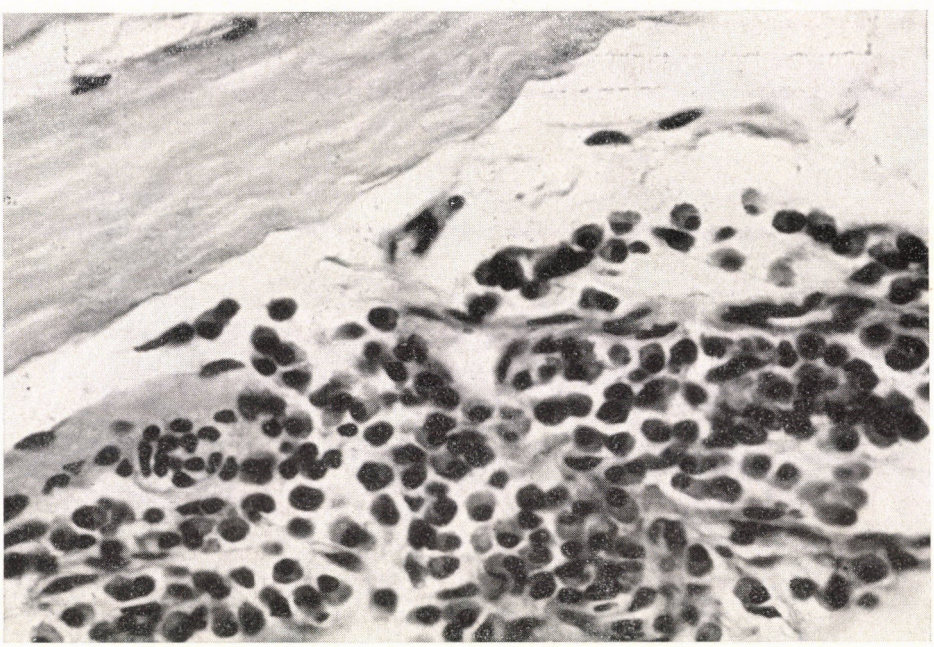

Fig. 1. Large collection of plasma cells in the muscle (H et E ×390)

The muscle biopsies made 10 months after the synovectomy showed slight lymphocytic infiltration of the perimysium and endomysium, otherwise the findings were within normal limits. There was no evidence of plasma cell infiltration.

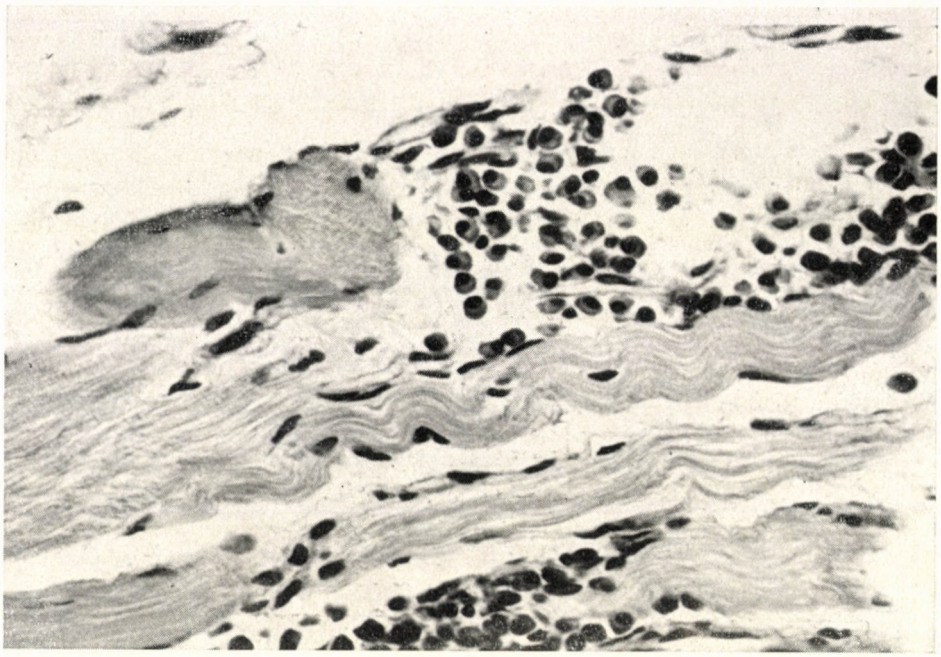

Fig. 2. Collections of plasma cells infiltrating muscle fibres (H et E ×265)

Discussion

The pure plasma cell myositis observed in our case has not been described among the muscle changes observed in rheumatoid arthritis. The patient had a rheumatoid disease of comparatively short duration. The synovectomy and first muscle biopsy were performed three months after the onset of symptoms. Operation so early in the course of this disease is uncommon, and in view of this there is a possibility that this type of abnormality may represent a very early involvement of the striated muscle. This possibility is supported by the fact that in early active rheumatoid arthritis the bone marrow may exhibit an increased number of plasma cells (FLEISCHAKER and LACHNIT [3], KLEIN and BLOCK [6]). This extensive plasma cell infiltration affecting the muscle fibres might be a morphological manifestation of a local immune reaction against the muscle proteins.

REFERENCES

1. BENEKE, G.: Die Reaktion des Muskelbindegewebes bei rheumatischen Erkrankungen. Z. Rheumaforsch **31,** Suppl. 2. (1972).
2. CURTIS, A. C. and POLLARD, H. M.: Felty's syndrome: its several features, including tissue changes, compared with other forms of rheumatoid arthritis. Ann. intern. Med. **13,** 2265 (1940).
3. FLEISCHAKER, H. and LACHNIT, V.: Blut und Knochenmarkbefunde bei chronischen Polyarthritiden und bei Feltyschen Syndrome. Wien. klin. Wschr. **53,** 189 (1940).
4. GARDNER, D. L.: The pathology of rheumatoid arthritis. Edward Arnold Ltd., London (1972).
5. GIBSON, H. J., KERSLEY, G. D. and DESMARAIS, M. H. L.: Lesions in the muscle in arthritis. Ann. rheum. Dis. **5,** 131 (1946).
6. KLEIN, H. and BLOCK, M.: Bone marrow plasmocytosis: a review of 60 cases. Blood **2,** 1034 (1953).
7. SOKOLOFF, L., WILENS, S. L., BUNIM, J. J. and MCEWEN, C.: Diagnostic value of histologic lesions of striated muscle in rheumatoid arthritis. Amer. J. med. Sci. **219,** 174 (1950).
8. STEINER, G. and CHASON, J. L.: Differential diagnosis of rheumatoid arthritis by biopsy of muscle. Amer. J. clin. Path. **18,** 931 (1948).
9. WITTENBROG, A.: Veränderungen der Muskulatur bei rheumatischen Arthritis. Dtsch. med. Z. **20,** 346 (1969).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (1—2), pp. 99—106 (1974)

DIFFERENTIAL DIAGNOSTICS AND SURGICAL INDICATIONS OF COLD THYROID NODULES

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(Received November 30, 1973)

The incidence of thyroid carcinoma, of thyroid tumours of potential malignancy and of thyroiditis was studied on the basis of the histological features of cold nodules observed in 300 thyroids from a population of a flat area with endemic goitre. Clinical parameters have been designed for use in the differentiation of cold nodules and in the timing of surgery.

Preoperative diagnosis of thyroid malignancy is still uncertain despite the superficial site of the gland which makes it readily accessible to examination. The introduction of radioiodine in the diagnostics of thyroid disease marks a turning-point in this respect (CASSEN et al. [6]). By the demonstration of cold areas in the thyroid, the surgical indications in nodular goitre for suspicion of malignancy have been greatly narrowed down. In addition, exact localization of the cold areas allowed the use of function-saving surgical techniques (ZUKSCHWERDT [45]). Yet, thyroid scintigraphy is not of conclusive diagnostic value. The absence of radioiodine uptake is not specific of tumours; other thyroid changes such as benign adenomas, cysts, thyroiditis, haemorrhages also give cold areas and cold nodules below a certain size, in general below 15 mm (THIEMANN and BAY [41]) are not detectable by scintigraphy. Therefore, the absence of radioiodine uptake serves merely as confirmatory evidence of malignancy in the case of clinical suspicion. It must also be noted that some thyroid tumours, even though a minority, are taking radioiodine.

There are two essential lines of approach to the objective of increasing the diagnostic reliability of scintigraphy. The first consists in developing the procedure still further by the use of other isotopes, in particular of ^{67}Gallium citrate (GREBE et al. [14]; MÜHE [31]; RICCABONA et al. [34]), of ^{32}Phosphorus (ACKERMANN et al. [1]), of ^{75}Selenium methionine (GREEN et al. [15]), etc., possibly in association with biopsy (GALVAN [12]; JANGINGER et al. [22]). The other is aimed at establishing clinical parameters on the grounds of the history, the surgical intervention, the histological features of the surgical specimen, thus enabling a closer differentiation of the surgical indications in the case of a cold nodule, the presence of which is regarded to-day as calling for surgery.

The present report deals with the histopathological features of the cold nodules and with the clinical parameters providing for a greater diagnostic accuracy of scintigraphy, on the basis of positive scans obtained in 300 patients from a population of an area with endemic goitre located in a flat district of North-East Hungary.

Material and method

A total of 277 females and 27 males observed in the period 1968 to 1972 were involved in the study. The scans were taken after administration of 25 to 30 μCi ^{131}I by a Siemens Nucleograph, at a skin-crystal distance of 25 to 30 cm, using a No. 3 collimator. The basic surgical technique was enucleation of the cold area. The later steps, i.e. modified or radical cervical dissection, depended on intraoperative or postoperative microscopic findings.

Results

A total of 44 cold nodules (15%) proved malignant; 28 of these were differentiated, 16 undifferentiated or sarcomatous. There was in addition a group of 34 patients (14%) with tumours of potential malignancy which still lack a consistent interpretation both pathologically and clinically (HUBER and FUCHSIG [20]; ZUCKSCHWERDT [47]; KEMINGER [23]). In fact, the follicular, trabecular and Hürthle-cell adenomas falling under this definition exhibit certain pathomorphological signs of malignancy, such as atypical cells, polymorphism, capsular infiltration, initial invasive growth, but their biological properties are still poorly understood. Opinions on their nature are therefore strongly divided. While being regarded by some clinicians as truly malignant tumours calling for radical surgery, they are considered by others merely as sources of potential malignancy warranting a more restrained therapeutic attitude. In the case of such tumours we refrained from radical surgery and carried out enucleation or subtotal resection, linked with a close postoperative follow-up. We also examined the incidence of thyroiditis accounting for the cold areas, and found thyroiditis, including its acute and chronic forms to be the source of the cold nodules in a total of 37 cases (12%) (Fig. 1). This was re-

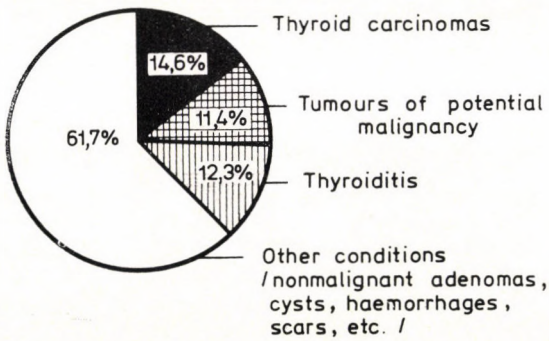


Fig. 1. Distribution of 300 cold nodules on a pathohistological basis

markable, as in 8 of the malignant cases microscopic changes characteristic of destructive autoimmune thyroiditis were found side by side with the malignant process.

On confronting our figures with the international data evaluated under various aspects, we find that the incidence of malignancy derived from large-scale surveys concerning a minimum of 200 cold nodules varies between 4.2 and 21.0%. The 14% malignancy rate found in the present study was slightly in excess of the average figures. The cause of this may have been that the patients had come from a district with endemic goitre and also because it has been sought to locate the area of biopsy as closely as possible (Table I).

As regards the microscopic features of malignant and potentially malignant tumours and of thyroiditis, we have found it remarkable that 1. notwithstanding the fact that the study concerned a population of a goitrogenous area, most tumours carried a comparatively favourable prognosis; 2. in the group of potential malignancy, the follicular, trabecular and Hürthle-cell

Table I

Malignization rate of thyroid cold nodules

Authors	Ref.	Number of cold nodules studied	Rate of malignization, per cent
Grosebeck	[16]	140	14.2
Horst et al.	[18]	154	26
Ernst and Günter	[10]	77	29
Zukschwerdt	[44]	219	21
Shimaoka and Sokal	[40]	88	10
Börner et al.	[3]	169	9.5
Johnson	[21]	223	10.4
Bay	[2]	650	16
Robinson et al.	[35]	102	22.5
Pörtener and Ungeheuer	[33]	159	15.7
Schacht and Mannfeld	[39]	176	5.7
Keminger	[23]	975	17.3
Knowlson	]25]	771	4.2
Kremer et al.	[26]	378	8.9
Röhrer et al.	]37]	803	5.1
Scazziga	[38]	300	10
Pietsch ét al.	]32]	564	6.7
McKenney et al.	[24]	1130	15.6
Brooks	[4]	210	19
Present material		300	14.6

adenomas had a practically equal share; 3. of the cases of thyroiditis, the majority presented an advanced destructive Hashimoto's thyroiditis, which implicates a potential preblastomatous state (Fig. 2).

1. The incidence of cold nodules was far lower in males than in females, while the rate of malignization was higher in males than in females. This is obvious even when the global figures for carcinomas and for the tumours of

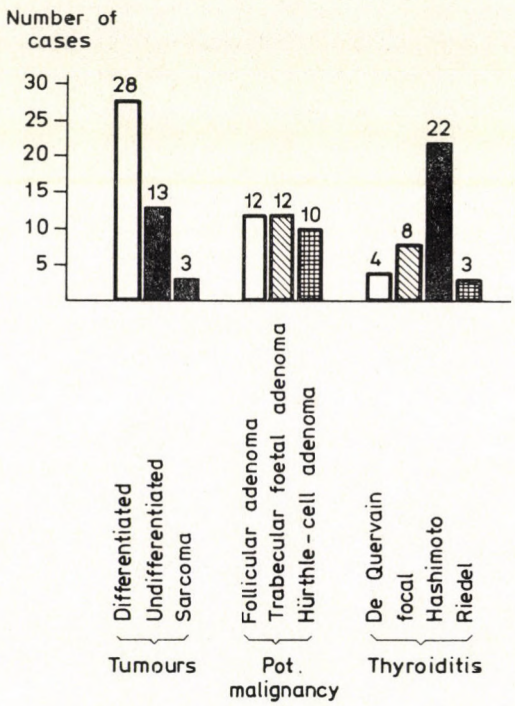


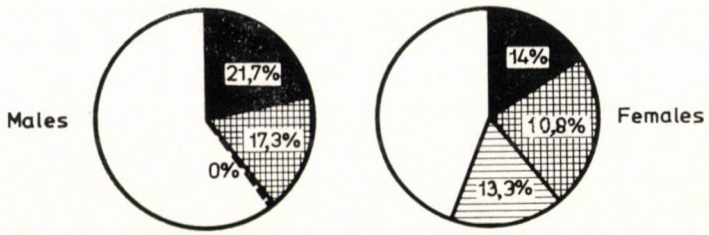
Fig. 2. Classification of tumours and inflammatory cleavages

potential malignancy are correlated with the total number of 23 and 277 cold nodules, respectively. The cold nodules in males were not numerous enough for a distribution according to age.

2. The rate of malignization of the cold nodules in females affected prevalently the young and the old age groups. The tumours in the former group were almost exclusively of differentiated, those in the latter group of undifferentiated, structure.

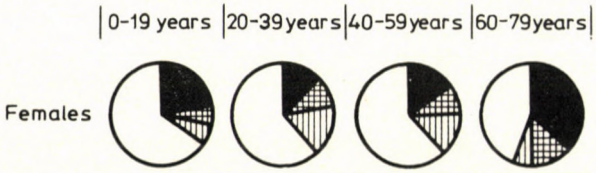
3. In middle-aged women, particularly in the period of menopause, thyroiditis accounted for a strikingly high proportion of the cold nodules (Figs 3, 4).

While in 20 of the 40 thyroid tumours the clinical features alone had been suggestive of malignancy, in 24 (55%) the final decision for surgery was made



	Males	Females
Number of cold nodules:	23	277
Carcinomas:	5	39
Pot. malignancy:	4	30
Thyroiditis:	0	37

Fig. 3. Sex distribution of carcinomas, tumours of potential malignancy and of thyroiditis



	0-19 years	20-39 years	40-59 years	60-79 years	
Number of cold nodules:	19	100	126	32	277
Carcinomas:	4	10	14	11	39
Pot. malignancy	1	11	11	7	30
Thyroiditis	1	16	18	2	37

Fig. 4. Age distribution of carcinomas, tumours of potential maignancy and of thyroiditis, in females

on the grounds of the scan. The clinical parameters to be discussed in the following have been found of major differential diagnostic value.

Clinical parametes

1. *Relationship between cold nodule and potential malignancy.* Potential malignancy confirmed by biopsy makes surgery imperative, and the intervention should be carried out without delay regardless of age or sex.

2. *Relationship between cold nodule and earlier X-ray irradiation of the cervical region.* It is well-known that in the case of radiotherapy applied to the cervical region for some non-malignant condition in childhood, thyroid carcinoma is found 10 to 15 years later three times as often as in non-irradiated age-matched subjects (DUFFY and FITZGREALD [7]; WINSHIP [42]; MELVIN et al. [28]). This implicates that with radiotherapy in the history, the presence of a cold

nodule calls for surgery regardless of age or sex. There were four such cases in the present series.

3. *Relationship between cold nodule and earlier radioiodine treatment.* Hyperthyroid laboratory animals develop malignant thyroid tumours after radioiodine treatment (EICHLER and HŐBEL [9]) and there have been a few observations to this effect also in humans. ZUCKSCHWERDT [46] reported on atypical adenomas formed after radioiodine treatment with microscopic features consistent with potential malignancy. In other words, development of cold nodules after radioiodine therapy raises the suspicion of carcinoma and makes surgery inevitable, regardless of age or sex.

4. *Age and sex relationships of the cold nodule.* In agreement with data in the literature, we regard the presence of a cold nodule in males, in view of the higher rate of malignization, as an absolute indication for surgery, regardless whether the patient belongs to a population of an endemic or of a non-endemic area. For the same reason, this also applies to cold nodules, particularly if solitary, in women of the infantile, juvenile and senile age groups. In middle-aged females the high incidence of thyroiditis makes an expectant attitude warrantable and surgery is refrained from, unless the nodule remains unchanged despite long-continued adequate treatment of thyroiditis.

5. *Relationship between cold nodule and its growth.* A fast, conspicuous growth of a cold nodule is suggestive of malignancy but it may be also due to bleeding into a benign adenoma. Though differentiation by biopsy is possible, a cold area of conspicious growth should at any rate be treated surgically.

6. *Relationship between cold nodule and recurrent goitre.* In the material of HUBER [19] and of EGLOFF [8] of supposedly non-malignant thyroid disease, 15 and 20%, respectively, of the surgically removed recurrent goitres displayed malignant changes. The occurrence of cold nodules in recurrent goitre, despite adequate thyroid hormone substitution, makes thus surgery imperative. In the present material there were 6 cases of malignancy connected with recurrent goitre.

7. *Relationship between cold nodule and ectopic thyroid tissue in the regional lymph nodes.* There are observations on occlusions of apparently normal thyroid follicles in cervical lymph nodes, mainly in cases of laryngeal carcinoma, but similar inclusions were noted also in cervical lymph node enlargement due to other causes. The pathological significance of these inclusions is nuclear. Though usually regarded as deposits of silent thyroid carcinomas (FISH and MOORE [11]; BUTLER et al. [5]; ZABRANSKY and HIRSCH [43]), they are considered non-malignant by other authors (GERARD-MARCHANT [13]; ROTH [36]; MEYER and STEINBERG [30]). In the case of a joint finding of ectopic lymphonodular thyroid tissue and of a solitary cold nodule, the probability of malignancy makes surgery advisable. No case of this kind has been encountered in the present series.

8. *Relationship between cold nodule and phaeochromocytoma*

The joint occurrence of phaeochromocytoma and of thyroid medullary carcinoma is frequent (HOENSCH [17]; MELVIN et al. [29]; LJUNGBERG [27]). High serum calcitonin levels in phaeochromocytoma make this association still more probable. At any rate, close investigation of the thyroid is obligatory in every case of confirmed phaeochromocytoma and detection of a cold nodule calls for surgery.

9. *Relationship between cold nodule and calcification*

Radiographic evidence of a homogeneous, coherent calcified area within the cold nodule generally reflects a non-malignant process. In contrast, stippled or patchy calcification may correspond to the calcified core of a psammoma characteristic of thyroid carcinoma (GREEN et al. 1972). In other words, the presence of patchy calcifications makes surgical removal of the cold nodule advisable.

In summary, taking into consideration the 15% malignization rate in our material, malignant tumours of the thyroid gland are by no means uncommon. The clinical parameters assembled in the present study add to the diagnostic value of scintigraphy and allow a closer differentiation of the cold nodule.

Acknowledgement

The authors are indebted to the staff of the Institute of Pathology, University Medical School, Debrecen (Head, Professor P. ENDES) for valuable help in the pathohistological investigations.

REFERENCES

1. ACKERMANN, N. B., SHANON, D. B. MARVIN, J. T.: IV. Int. Goitre Conference, London (1960).
2. BAY, V.: Langenbecks Arch. klin. Chir. **316,** 101 (1966).
3. BÖRNER, W., LAUTSCH, M., MOLL, E., ROMEN, W.: Med. Welt **17,** 892 (1965).
4. BROOKS, J. R.: Amer. J. Surg. **125,** 477 (1973).
5. BUTLER, J. J., TULINIUS, H., IBANEZ, M. L., BALLATYNE, A. J., CLARK, L.: Cancer **20,** 103 (1967).
6. CASSEN, B., CURTIS, L., REED, C.: Nucleonics **6,** 78 (1950).
7. DUFFY, B. J. jr., FITZGERALD, P. J.: Cancer **3,** 1018 (1950).
8. EGLOFF, B.: Schweiz. med. Wschr. **91,** 424 (1961).
9. EICHLER, O., HŐBEL, M.: Langenbecks Arch. Klin. Chir. **311,** 209 (1965).
10. ERNST, H., GÜNTER, S.: Strahlentherapie **119,** 584 (1962).
11. FISH, J., MOORE, R. M.: Ann. Surg. **157,** 212 (1963).
12. GALVAN, G.: Dtsch. med. Wschr. **95,** 1631 (1970).
13. GERARD-MARCHANT, R.: Arch. Path. **77,** 633 (1964).
14. GREBE, S. F., SCHÖN. H., STECKENMESSER, R., HEGER, N.: Langenbecks Arch. Klin. Chir. **329,** 233 (1971).
15. GREEN, W., SENTURIA, H., PACKMAN, R., RICHARDS, F.: J. Amer. med. Ass. **211,** 1265 (1972).

16. Grosebeck, H. P.: Cancer **12,** 1 (1959).
17. Hoensch, H.: Dtsch. med. Wschr. **96,** 126 (1971).
18. Horst, W., Petersen, I., Thiemann, Kl. J., Zukschwerdt, L.: Dtsch. med. Wschr. **85,** 711 (1960).
19. Huber, P.: Krebsarzt **11,** 14 (1956).
20. Huber, P., Fuchsig, P.: Wien. klin. Wschr. **70,** 876 (1958).
21. Johnson, J. R.: Calif. Med. **102,** 194 (1965).
22. Janginger, Th., Finsterer, H., Splesberg, F., Erpenbeck, R., Pichlmayer, H.: Langenbecks Arch. klin. Chir., Supp. 199 (1972).
23. Keminger, K.: Wien. klin. Wschr. **83,** 510 (1971).
24. McKenney, J. F., Petty, F. C., Peterson, R. F.: Surg. Clin. N. Amer. **52,** 383 (1972).
25. Knowlson, G. T. G.: Brit. J. Surg. **58,** 253 (1971).
26. Kremer, K., Gisbertz, K. H., Höhmann, H., Schacht, U.: Zbl. Chir. **96.** 356 (1971).
27. Ljungberg, O.: Acta path. microbiol. scand., Suppl. **231,** 1 (1972).
28. Melvin, A. B., Miller, M. J., Horn, R. C. jr.: Amer. J. Surg. **118,** 764 (1969).
29. Melvin, K. E. W., Miller, H. H., Tashjian A. H. jr.: New Engl. J. Med. **285,** 1115 (1971).
30. Meyer, J. S., Steinberg, L. S.: Cancer **24,** 302 (1969).
31. Mühe, E.: Langenbecks Arch. klin. Chir. **329,** 232 (1971).
32. Pietsch, P., Dabels, J., Schwartz, K. D.: Zbl. Chir. **97,** 1265 (1972).
33. Pörtener, J., Ungeheuer, E.: Med. Welt. **18,** 1302 (1967).
34. Riccabona, G., Scholz, K., Bauer, H.: Langenbecks Arch. Klin. Chir. **329,** 234 (1971).
35. Robinson, E., Horn, Y., Hochmann, A.: Surg. Gynec. Obstet. **123,** 1024 (1966).
36. Roth, L. M.: Cancer **18,** 105 (1965).
37. Röhrer, H. D., Rudolph, H., Wunsch, W., Griep, J.: Langenbecks Arch. Klin. Chir., Suppl.191 (1972).
38. Scazziga, B. R.: Praxis **61,** 1038 (1972).
39. Schacht, N., Mannfeld, N.: Dtsch. med. Wschr. **95,** 1521 (1970).
40. Shimaoka, K., Sokal, J. E.: Arch. intern. Med. **114,** 36 (1964).
41. Thiemann, Kl. J., Bay, V.: Langenbecks Arch. Klin. Chir. **322,** 1223 (1968).
42. Winship, T.: Pediatrics **18,** 459 (1956).
43. Zabransky, S., Hirsch, W.: Münch. med. Wschr. **115,** 542 (1973).
44. Zukschwerdt, L., Bay, V.: Wien. med. Wschr. **113,** 823 (1963).
45. Zukschwerdt, L.: Therapiewoche **24,** 1189 (1964).
46. Zukschwerdt, L., Bay, V., Gusek, W.: Med. Welt. **17,** 745 (1966).
47. Zukschwerdt, L.: Z. ärztl. Fortbild. **19,** 25 (1969).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (1—2), pp. 107—114 (1974)

MODEL MYOCARDITIS IN THE RAT: STUDY OF THE INFLUENCE OF MUSCULAR EXERCISE

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(Received December 11, 1973)

Myocardial changes similar to those of human interstitial myocarditis were induced in adult rats by a diet containing lauric acid ethylester. Muscular exercise was found to aggravate the lesion. On comparison of the ECG features with the microscopic findings, the ECG proved a reliable indicator of the clinical course.

An attempt has been made to produce an experimental of human myocarditis and myocardial changes were successfully induced in rats immediately after weaning. The process was followed up by serial ECG recordings which then were confronted with the microscopic features [8].

In small laboratory animals, feeding of a fat-rich diet results in a variety of lesions affecting the myocardium, the walls of the large vessels, the kidney and the liver. The most consistent changes reported in the literature include focal necrosis and haemorrhages of the myocardium and the renal parenchyma, sclerosis of aorta and coronaries, fatty degeneration of the liver [2, 4, 5, 9, 10, 12]. In the chronic type of such lesions, fibrosis and calcification may ensue. Their severity is increased by choline deficiency [3]. It was difficult to produce changes of this kind in adult or large animals, as these are less sensitive to the diet. The cardiovascular lesions, if any, were insignificant even in the case of experiments and were found the most extensive and the most rapidly arising lesions after the use of the synthetic triglycerides lauric acid. ethyl laurate and ethyl caprylate [11]. Triglycerides primarily affect the myocardium, lesions of other organs (major vessels, kidney, liver) being insignificant and even likely to remain absent in acute experiments. The myocardial lesions thus produced are regarded by numerous authors as similar in their features to human interstitial myocarditis [7, 9, 10], a view supported by the results of our earlier studies [8].

The experimental changes induced in young rats take a rapid course and end fatally in 2 or 3 weeks, a period too short to permit a closer observation of the clinical features. Moreover, the laboratory investigations are made difficult by the small body weight and the minute amounts of blood available. Finally, owing to the high vulnerability of young animals, the diet invariably results in a rapid fatal course, consequently, the animals die before attaining

the stage of myocardial fibrosis, still less that of calcification. This has prompted us to adapt the procedure to adult rats. In this way a process of more protracted course was obtained, allowing to test the influence of muscular exercise on the lesions thus induced.

Attention was also given to the ECG abnormalities associated with the myocardial changes. Laboratory investigations likely to provide information on the extent of tissue destruction were also carried out. Finally, the data thus obtained were confronted with the microscopic features.

Material and method

40 Wistar rats of both sexes weighing between 170 and 220 g were studied. Four groups of 10 animals each were formed. The animals of groups I and II received 35% ethyl laurate admixed to standard rat food, those of groups III and IV were kept on standard food. Water was allowed ad libitum. 10 animals of the groups fed the cardiopathogenic diet and 10 of those fed normal food (groups II and III) were subjected to swimming 3 to 5 times weekly. From the 3rd month of the experiments onward, under ether anaesthesia ECG recordings were made twice weekly with a six-channel Hellige apparatus, at paper speeds of 50 and 100 mm sec for 30 sec to 1 min. Serum potassium, SGOT, ESR and WBC were checked at monthly intervals and prior to the sacrifice of the animals [1]. Compared with the other groups, the animals of group II (diet + exercise) were retarded in their development, they failed to gain weight, their tolerance to muscular exercise gradually deteriorated and although at first their performance was virtually limitless in the same manner as in the normal rats, later they grew more and more fatigable. 10 to 14 days after being started on the diet 30 to 40 min, later 8 to 12 min of the exercise resulted in complete exhaustion. 4 months after the start of the studies the animals were killed by exsanguination. The heart, one kidney and liver of each animal were processed for microscopic study.

Results and discussion

The results of the ECG studies have been tabulated (Table I). The deviations between the normal and abnormal ECG features were tested for significance in each group by the λ^2-test. The differences between the group which

Table I

Group	Changes in heart rate	Low voltage	Nodal rhythm	Es and other arrhythmias	AV block	Lesion	Necrosis	Electrical alternans	ECG total	Abnormal ECG, No.	Abnormal, per cent
I.											
D	—	4	6	6	12	—	2	—	140	30	21.4
II.											
D + E	7	4	6	8	21	4	5	11	118	66	55.9
III.											
E	5	—	4	2	7	—	—	—	122	18	14.8
IV.											
C	4	—	3	3	6	—	—	—	145	16	11.1

D = diet E = exercise C = controls

had diet only and the controls were found significant ($p < 5\%$), and those between the group which had muscular exercise in addition to the diet and the controls were found highly significant ($p < 0.1\%$). A similar degree of significance was found between the group subjected to diet and swimming and the animals which had muscular exercise only and those which had diet only. This indicated that the pathogenicity of the diet was greatly enhanced by muscular exercise.

In conformity with published observations [6] we also found that changes in heart rate, in voltage as well as in impulse production (e.g. nodal rhythm), were not abnormal, such modifications being common in normal animals. It was particularly striking to find complete or incomplete AV block in numerous controls. A transitory block may be caused by anaesthesia or the stress factors involved but its persistence must, however, be regarded as definitely abnormal and attributed to the involvement of the conduction system. This implicates the necessity for repeated ECG recordings over a sufficiently long period. Other remarkable features included signs of lesion and necrosis, in the first place in group II (Figs 1, 2), as also electrical alternans which was

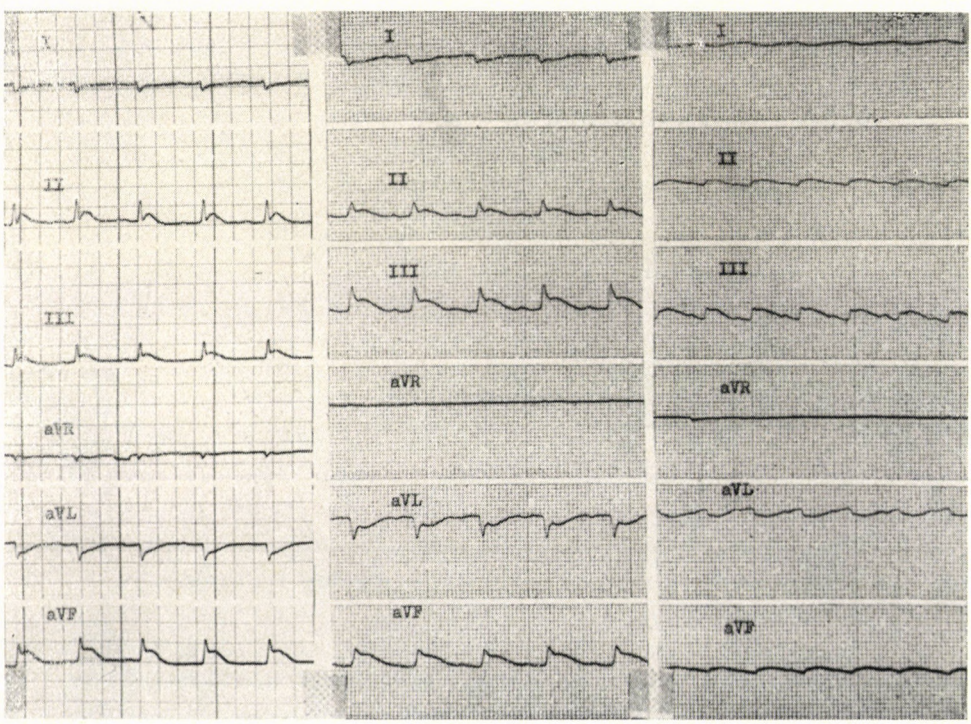

Fig. 1. ECG leads of the same animal in the 10th, 13th and 15th week of the experiment. Note the progressive tendency of the ST-T abnormality and the decline in voltage. (Paper speed, 100 mm/sec)

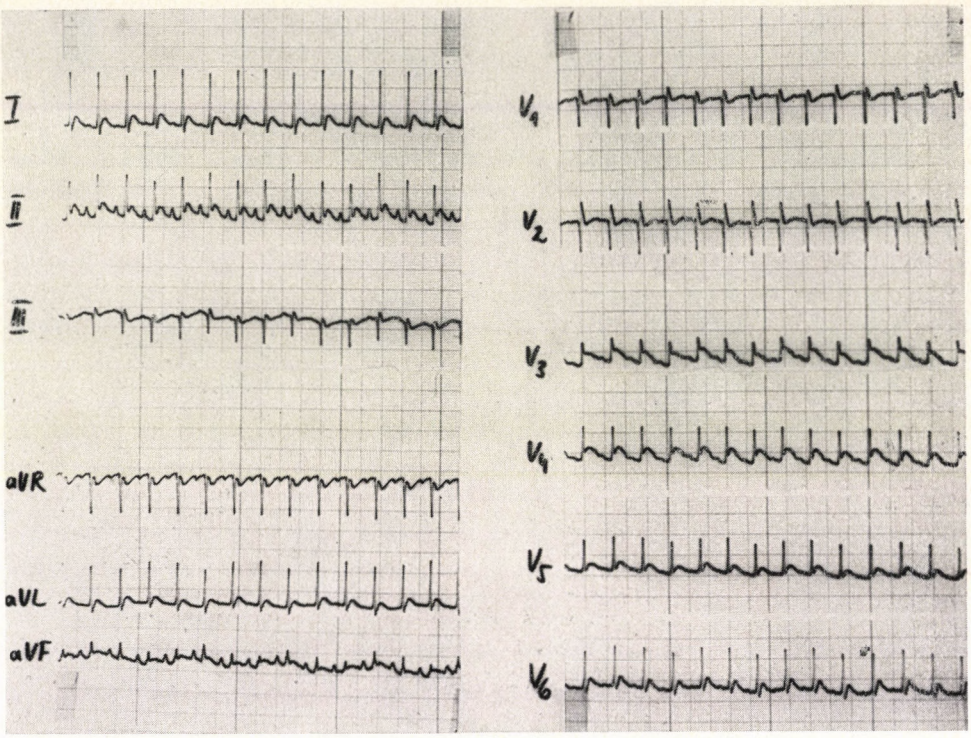

Fig. 2. ECG of a rat in the 16th week of the experiment. The abnormal Q in leads III, V_1 and V_2 points to necrosis. (Paper speed, 100 mm/sec)

found in this group alone (Fig. 3) and generally proved permanent in the individual animals, indicating a myocardial damage similarly as in humans.

The extent of changes was closely reflected by the changes in body weight. In group IV (controls) there was an average gain of 65 g, in group I (diet only), of 30 g; in group III (exercise only) likewise of 30 g; in the course of 4 months, in opposition to group II (diet and exercise), where there was an average loss of 6 g per animal. Heart weight was also different in the individual groups, i.e. 1125 mg for group IV (controls); 1465 mg for group I (diet); 1574 mg for group II (diet + exercise); 1200 mg for group III (exercise). The ratio, heart weight per 100 g body weight, showed a significant difference in favour of group II, as a sign of cardiac hypertrophy having developed in the course of the experiments.

It was sought to gain information from laboratory tests on the extent of tissue destruction. The results of these studies were, however, in the normal range. The cause of this may lie in the timing of the samplings which may have been performed when the destructive lesions were not extensive enough to produce any significant change in SGOT or of ESR. Samplings at closer inter-

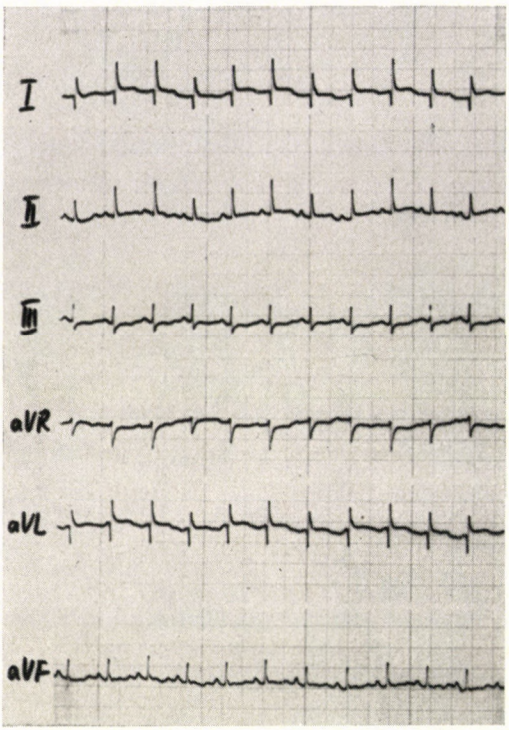

Fig. 3. Electrical alternans in the 13th week of the experiment

vals and use of more sensitive procedure might prove more informative. It is to be noted that human myocarditis also lacks typical laboratory signs.

Necropsy revealed no gross changes. Even microscopic ones were confined to groups I and II, i.e. to the animals having been fed ethyl laurate. In groups IV (controls) and III (normal diet with muscular exercise) no myocardial abnormalities were seen. The most marked and most extensive lesions involving both ventricles and the septum in the form of an interstitial infiltration by lymphocytes, histiocytes and fibroblasts were found in the animals of group II. The consecutive parenchymal changes included a disappearance of muscular striation, swelling, increased eosinophilia. Fragmentation of the myocardial fibres was also a common finding. At sites, the interstitium contained hypertrophic connective tissue of a loose delicate fibrous structure (Figs 4, 5).

In the animals of group I, the changes were similar but less marked. The interstitial changes were of focal character and the parenchymal lesions of lesser severity (Fig. 6). In the animals treated with ethyl laurate, kidney and liver changes were also present in the form of thickening and fusion of the glomerular basement membrane, homogeneous eosinophilic material filling

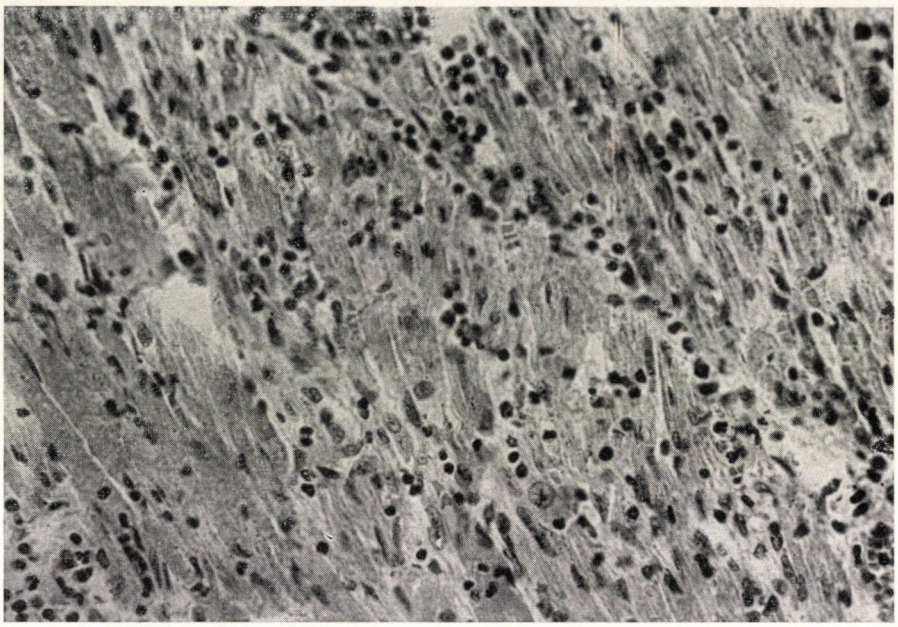

Fig. 4. Extensive inflammatory infiltration of interstitial tissue. Myofibrillar fragmentation; the normal structure of the sarcoplasma has disappeared (H. E. ×140)

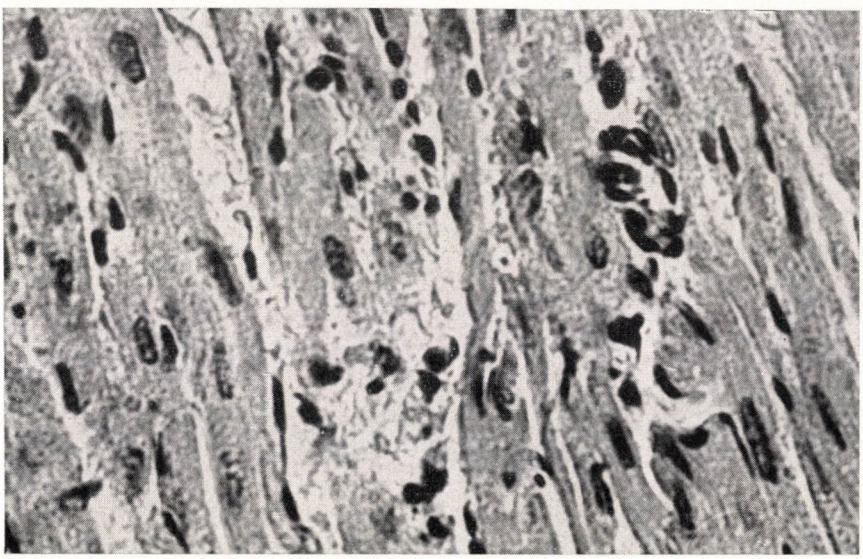

Fig. 5. Between the oedematous myofibrillar bundles there are fibroblasts and round cells. The broadened interstitium is occupied by hypertrophic connective tissue of loose fibrous structure (H. E. ×240)

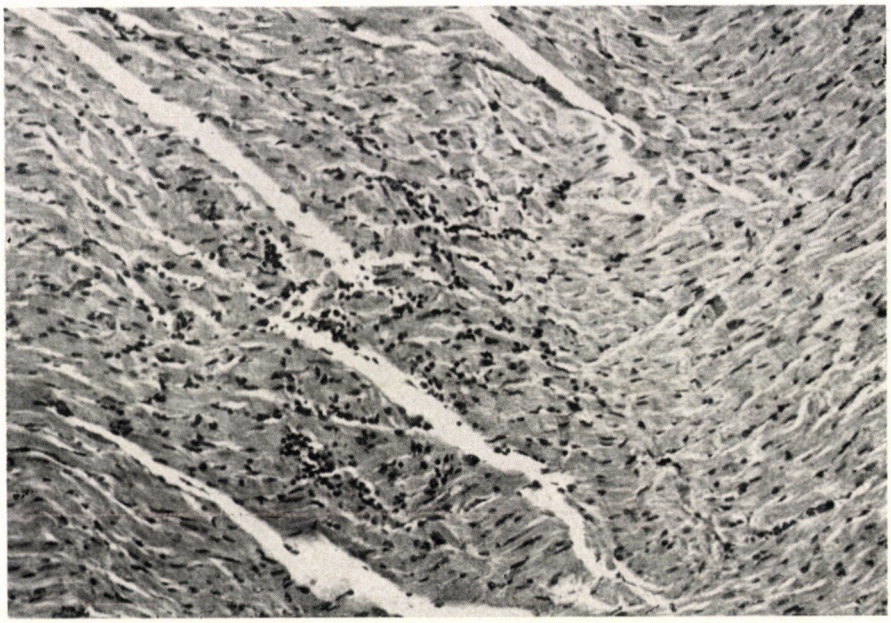

Fig. 6. Focal round-cell infiltration in interstitial tissue

the tubular lumen in the kidney, and parenchymal degeneration and hyperplasia of Kupffer's cells, in the liver.

The clinical signs were in harmony with the microscopic findings. The most extensive myocardial lesions were found in group II, with loss of body weight, poor exercise tolerance and prevalence of persisting ECG changes. These features corresponded to interstitial myocarditis. The inflammatory process was of acute or subacute character with early signs of fibrosis which had not yet progressed to the chronic stage. The lesions produced in the present study exceeded in severity those reported by other workers, and ECG abnormalities were likewise more frequent. The cause of this may be sought in the heavy muscular exercise associated with the diet.

The extent and severity of the abnormalities noted in group I were consistent with published evidence [8, 9, 10], they were, however, not sufficient for a closer study of the process.

The renal and hepatic changes observed in the animals fed ethyl laurate were unrelated to the myocardial lesion. This implicates that the myocardial changes were due to a direct cardiopathogenic effect of the triglyceride rather than to an impairment of renal or hepatic function or to any other functional deterioration [11].

Acknowledgements

We are indebted to Miss E. Tákos and Mrs. I. Molnár for laboratory assistance and to Miss E. Molnár for statistical analyses.

REFERENCES

1. BÁLINT, P.: Klinikai laboratóriumi diagnosztika. Budapest (1962).
2. ENGEL, R. W., SALMON, W. D.: J. Nutr. **22,** 109 (1941).
3. GRIFFITH, W. H., WADE, N. J.: J. biol. Chem. **131,** 567 (1939).
4. HARTROFT, W. S., RIDOUT, J. H.: Amer. J. Path. **27,** 951 (1951).
5. HARTROFT, W. S.: Circulation **10,** 588 (1954).
6. KENEDI, I.: Acta physiol. Acad. Sci. hung. **34,** 29 (1968).
7. KESTEN, H. D., SALCEDO, J. JR., STETTEN, D. JR.: J. Nutr. **29,** 171 (1945). (cit.)
8. SCHWARCZMANN, P., DEMETER, J., MAGYAR 8..: Acta med. Acad. Sci. hung. **29,** 261 (1972.)
9. SELYE, H.: Experimental Cardiovascular Diseases I—II. Springer Verlag, Berlin—Heidelberg—New York (1970).
10. STETTEN, W. JR., SALCEDO, J. JR.: J. Nutr. **29,** 167 (1945). (cit.)
11. WILGRAM, G. F., HARTROFT, W. S., BEST, C. H.: Brit. med. J. **3,** 1 (1954).
12. WILGRAM, G. F., HARTROFT, W. S., BEST, C. H.: Science **119,** 842 (1954).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (1—2), pp. 115—118 (1974)

IMMUNOGLOBULINS ON THE SURFACE OF LYMPHOCYTES IN AUTOIMMUNE DISEASE

II. Lymphocytotoxins on the surface of lymphocytes

By

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(Received December 18, 1973)

The influence of SLE-associated lymphocytotoxins was studied on lymphocytes bearing immunoglobulins on their surface. Attachment of lymphocytotoxin to normal lymphocytes is indetectable by indirect immunofluorescence. Since the binding is not inhibited by pretreatment with anti-human immunoglobulin, the lymphocytotoxins attach to T-lymphocytes too. Exposure to trypsin results in a release of the bound lymphocytotoxins, and these were demonstrated in 20% of subjects with autoimmune disease. The relationships between the cells bearing immunoglobulin on their surfaces and B-lymphocytes are discussed.

The sera of most SLE patients reveal lymphocytotoxins of the 19 S IgG type, their presence being related to the activity of the process [1, 4, 5, 7, 8, 9, 11]. The destructive action of these lymphocytotoxins is not confined to allogeneic lymphocytes but may be directed at autologous lymphocytes too [5, 11]. Addition of complement results in a lysis of the majority of the lymphocytes of SLE patients [6, 10] as a sign of the presence of membrane-bound lymphocytotoxins. On the evidence of our studies, the periods of activity in autoimmune disease are associated with a relative increase in the number of B-lymphocytes, in contrast to the periods of remission in which the B : T cell proportion is normal. The relative increase in the B-cell count is due to a decrease in the absoulute T-cell count [2, 3]. Of the various interpretations of T-cell depletion, their utilization or fixation in the target tissues seems to be the most convincing. We have, however, to consider the possibility that the lymphocytotoxins, at least in SLE, as a result of their binding to the T-lymphocyte surface, make the counts of SIg cells appear larger than they are in reality, and therefore the increase in the number of the B-cells is only apparent. The aim of the present study was to clarify this question.

Material and methods

Lymphocytes of 25 subjects with autoimmune disease in the period of exacerbation (19 cases of SLE, 5 of rheumatoid arthritis, one case of autoimmune disease of undefined character) and of 12 normal controls were studied. For their isolation and the demonstration of

the lymphocyte surface immunoglobulins the procedure described earlier [3] was used. The count of immunoglobulin-bearing cells (SIg) was obtained by adding the numbers of the IgG-, IgA- and IgM-bearing cells.

Trypsin treatment. For elution of membrane-bound lymphocytotoxins, 0.25% trypsin in TC-199 was used. 1 ml of the cell suspension (10^6 lymphocytes per ml) was incubated with an equal amount of the trypsin solution at 37°C for 30 min, then washed twice with the medium, centrifuged at 200 g for 10 minutes, and incubated at 37°C for 6 hours. After trypsinization, the surface immunoglobulins are newly formed in 6 hours. Incubation was followed by washing of the cells and examination of the surface immunoglobulins.

Lymphocytotoxin treatment. SLE serum of known lymphocytotoxin titre was used after inactivation at 56°C for 30 min. 1 ml (10^6 cells per ml) of the lymphocyte suspension was incubated with an equal amount of undiluted serum at laboratory temperature for 1 hour. After incubation the cells were washed three times and the surface immunoglobulins were examined.

Pretreatment of the lymphocytes with anti-human IgG: 1 ml (10^6 cells/ml) of the control lymphocyte suspension was incubated with 1 ml anti-human immunoglobulin (IgG + IgA + IgM) at 4°C for 30 min and washed three times with the medium. Then the cell suspension was incubated with 1 ml of lymphocytotoxin-containing serum at laboratory temperature for 1 hour and washed. The surface immunoglobulins were examined before and after treatment with anti-human immunoglobulin and after lymphocytotoxin treatment.

Results

In 20% of the patients (group A), trypsin treatment was followed by a significant (17%) fall in the SIg-cell count (Table I). No significant change

Table I

Influence of trypsin-treatment on the SIg-cells of autoimmune patients (SIg cells, per cent ± SD)

	Before trypsin treatment	After trypsin treatment
Controls	26.0±6.8	25.5±5.0
Autoimmune patients (Group A)	48.0±9.9	30.3±5.0
Autoimmune patients (Group B)	43.8±10.2	40.5±4.4
Autoimmune patients (total)	42.3±8.1	36.1±3.9

was demonstrated in 80% of the patients (group B) and in the controls. The reduction mainly affected the IgG-bearing cells, whereas the numbers of IgA- and IgM-positive cells remained practically unchanged.

The lymphocytotoxin-containing SLE sera produced an increase in the number of SIg cells derived from normal donors in 75% (Table II). In 3 cases, the lymphocytotoxin-induced increase in the SIg-cell count was not significant, in the other 9 cases it ranged between 10 and 78%. The mean SIg-cell proportion was 53.2% after treatment with serum. In most cases, a marked increase was demonstrable in the proportion of IgG-bearing cells.

Pretreatment with anti-human immunoglobulin blocked the binding of FITC-labelled anti-human immunoglobulin, and in consequence practically

Table II

Binding of SLE-lymphocytotoxin to lymphocytes of normal subjects (SIg cells, per cent ± SD)

Before treatment with SLE serum	After treatment with SLE serum
26.0±6.8	53.2±8.8

no SIg cells occurred. Pretreatment with lymphocytotoxin-containing serum resulted, however, in a 58.3% increase in the number of demonstrable SIg cells (Table III).

Table III

Influence of anti-human immunoglobulin pretreatment on the binding of lymphocytotoxin

	SIg cells per cent ± SD
Control	26.0±6.8
After AHIG* treatment	1.8±0.2
Effect of SLE serum after AHIG pretreatment	58.3±17.5

* Anti-human immunoglobulin.

Discussion

The lymphocytotoxins occurring in SLE attach to autologous lymphocytes. This is indicated by the observation that the appearance of lymphocytotoxins is followed by leukopenia [1]. Exogenous (rabbit) complement produces a lysis of lymphocytes of SLE patients even in the absence of additional lymphocytotoxin-containing serum; this is indicative of the presence of membrane-bound lymphocytotoxins [6, 10]. Binding of lymphocytotoxin to normal lymphocytes is demonstrable by immunofluorescence. Treatment with lymphocytotoxin resulted in a significant increase in the number of SIg cells in 75% of the cases. Pretreatment with anti-human immunoglobulin failed to block the binding of lymphocytotoxins. From this it may be inferred that the lymphocytotoxins are not of antiglobulin character and presumably attach to B and to T lymphocytes. Although after binding to lymphocytes, in 75% of the controls the number of SIg cells increased twofold, in other words, the B cells acccount for not more than approximately 50% of the SIg cells, on the lymphocytes of autoimmune patients binding of this extent was unfrequent. After trypsin treatment the decrease in the number of SIg cells was not more

than 6.2%, thus the B lymphocytes accounted for 35.1% of the 42.3% SIg-cells. This seeming contradiction may have various causes. First of all, lymphocytotoxins are mainly found in SLE but not in the same amounts in all active cases. Moreover, they seem to have a strong affinity to heterologous, even though normal, lymphocytes. It also seems likely that the lymphocytotoxin-coated lymphocytes are destroyed too rapidly to be demonstrable in their totality.

In previous studies [2, 3] we have discussed the possible causes of the increase in the proportion of B cells and of T cell depletion in autoimmune diseases. On the evidence of our findings, in most cases the increase in the number of SIg cells due to circulating lymphocyte-bound lymphocytotoxins was not significant, in other words the SIg cells are in fact B lymphocytes. Despite this fact, in occasional cases the increase in the proportion of SIg cells as a result of binding of lymhocytotoxins to the lymphocyte membrane may actually give the appearance of an increased B cell population, a number of T cells appearing as B cells.

REFERENCES

1. Butler, W. T., Sharp, J. T., Rossen, R. D., Lidsky, M. D., Mittal, K. K., Gard, D. A.: Arthr. and Rheum. **15,** 231 (1972).
2. Gergely, P., Szegedi, Gy., Fekete, B., Szabó, G., Petrányi, Gy.: Lancet **1,** 482 (1973).
3. Gergely, P., Szegedi, Gy., Szabó, G., Fekete, B., Petrányi, Gy.: Acta med. Acad. Sci. Hung. (in press).
4. Mittal, K. K., Rossen, R. D., Sharp, J. T., Lidsky, M. D., Butler, W. T.: Nature (Lond.) **225,** 1255 (1970).
5. Stastny, P., Ziff, M.: Arthr. and Rheum. **13,** 350 (1970).
6. Stastny, P., Ziff, M.: Arthr. and Rheum. **14,** 734 (1971).
7. Stastny, P., Ziff, M.: Lancet **1,** 1239 (1971).
8. Stastny, Ziff, M.: Clin. exp. Immunol. **8,** 543 (1971).
9. Stenszky, E.-né, Szegedi, Gy., Aszódi, L., Petrányi, Gy.: Haematologia (in press).
10. Stenszky, E.-né, Szegedi, Gy.: Personal communication.
11. Terasaki, P. I., Mottironi, V. D., Barnett, E. V.: New Engl. J. Med. **283,** 724 (1970).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (1—2), pp. 119—129 (1974)

STABILITY OF ^{131}I-THYROXINE AND OF ^{131}I-TRI-IODOTHYRONINE: THE INFLUENCE OF RADIOLYTIC DISINTEGRATION ON CERTAIN DIAGNOSTIC TESTS

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(Received January 15, 1974)

The blood-protein fractions responsible for the transport of thyroid hormones (TBG, TBPA, TBA) were assayed for their thyroxine-binding capacity in the serum of the same control subject over a one-year period, by a procedure based on the isotope-dilution technique. In the dilutions of $^{131}I—T_4$ (Amersham RCC) required for the procedure, the ratio $^{131}I—T_4$: $^{131}I—T_3$ was measured in every case. Parallel with the accumulation of $^{131}I—T_3$ resulting from deiodination of $^{131}I—T_4$, the binding capacity of the individual fractions was found to have shifted from TBG to TBPA. The fact that, in contrast to the principle of the isotope- dilution technique, the labelled substance and the non-radioactive T_4 were partly different, suggests that the measurements of radioactivity do not reflect the true binding conditions of T_4.

Successive batches of $^{131}I—T_3$ were examined in the same manner, and the values of the Hamolsky test were determined in the same serum. The figures displayed little variations and $^{131}I—T_3$ was also found significantly more stable than $^{131}I—T_4$.

Thus, the Hamolsky test was found to represent a fairly reliable indicator of thyroid function, in contrast to measurement of the T_4-binding capacity of the blood protein fractions by the isotope-dilution technique, the results of which are uncertain and therefore inconclusive in both clinical and therapeutic respects. It is suggested that the $^{131}I—T_4$ serving for the assays should be supplied as a substance and diluted before use, but not later than a few days after preparation. The advantages of doublet tagging are pointed out.

The transport of thyroid hormones in the blood serum is effected by three protein fractions: thyroxin-binding globulin (TBG), thyroxine-binding prealbumin (TBP), and thyroxine-binding albumin (TBA), forming together the thyroxine-binding protein (TBP) (FRIEDRICH et al. [1]). Information on the degree of hormone supply of these protein fractions and on their further saturability with exogenous hormones offers important clues to the diagnosis of thyroid disease.

On the evidence of data in the literature, thyroxine (T_4) has the strongest affinity to TBG, this fraction having a 66% share in its transport. It attaches less firmly to TBPA transports approximately 30%. Of the three fractions, TBA has the slightest thyroxine-binding capacity and accounts for no more than 10 to 15% of the transport (WAHNER and WALSER [2]). The other highly active thyroid hormone, tri-iodothyronine (T_3) differs from T_4 in its binding affinity. Though it is bound to all three protein fractions, this attachment is much

weaker than that of T_4 which implicates that T_4 is capable of displacing T_3 from its binding site to some other site of slighter affinity, for instance from TBG to TBPA (LARSEN [3]). On the other hand, all binding sites which have not been occupied by endogenous T_4 can be saturated with T_3 without involving its displacement by T_4.

These are the principles underlying the fractional (LEMARCHAND-BÉRAUD et al. [4]) and the global (HAMOLSKY et al. [5, 6]) measurements.

In an earlier study we have examined the hormone-binding capacity of the individual protein fractions (TBG, TBPA, TBA) by means of the isotope-saturation method (REVICZKY and SZÁNTÓ [7]), commenting also on the LEMARCHAND-BÉRAUD procedure [4] in the light of our experience. On following up the T_4-binding capacity of the blood proteins in sera of control subjects after a long drug-free period, the quantitative data were found to scatter more widely than expected from the values for serum organic iodine (PBI). In view of the wide scatter, we extended our studies to hypothyroid, euthyroid, and hyperthyroid patients. This time, some degree of correlation was found between the mathematical means and the hyper- or hypofunction of the thyroid, there was, however, a substantial overlap between the groups.

The problems of the method itself have been amply dealt with in the literature. The shift in pH, the individual batches of filter paper, the differences in the temperature of denaturation have been all examined as potential factors which might affect the results of the tests, the possible artifactitious character of TBPA having been also given consideration (DAVIS and GREGERMAN [8] OPPENHEIMER et al. [9]; TATA [10]).

In view of these facts it was expected that examination of the stability of radioactive T_4 should throw light on the causes of the wide divergence between our figures and those of LEMARCHAND-BÉRAUD et al. [4] concerning the binding capacities. Considering that, as pointed out above, T_4 attaches to the protein fractions in other proportions than does T_3, that it is capable of dispacing T_3 from individual protein fractions, furthermore that, as confirmed by REVICZKY et al. [11], as a hyperiodinated product it is highly liable to deiodination, it was safe to assume that accumulation of T_3 resulting from deiodination provides a major source of error. We have therefore studied the following points.

1. The amount of thyroxine and of tri-iodothyronine contained in the diluted $^{131}I—T_4$ samples.

2. The correlation between the modifications of the thyroxine content of $^{131}I—T_4$ and the activity of TBG and TBPA.

3. Is the tri-iodothyronine content of the diluted $^{131}I—T_3$ subject to fluctuations similar to those of $^{131}I—T_4$?

4. Are the values for the Hamolsky test modified in proportion to the possible disintegration of $^{131}I—T_3$?

Material and methods

I. Radioactive solutions. $^{131}I-T_4$ in 50% propylene glycol (Amersham RCC); mean concentration 6.5 μg T_4/ml; specific activity, 35 μC/μg. Stored at +4°C, then diluted with physiological saline to 0.2 μg/ml T_4.

II. Inactive thyroxine solution prepared from Callbiochem B/grade thyroxine sodium with physiological saline. T_4 concentration = 1.06 μg/ml, corresponding to 0.68 μg I/ml. Stored at +4°C.

II/a. Saturating solution, a mixture of radioactive and inactive thyroxine, made up to 0.80 μg iodine per ml (0.12+0.68 μg T_4-iodine).

III. $^{131}I-T_3$ in 50% propylene glycol. Mean concentration, 8.9 μg/ml; specific activity, 34 μC/μg. Stored at +4°C. Approximately hundredfold dilutions were prepared with physiological saline, containing 0.4 to 0.8 μg substance per 100 ml.

IV. The blood serum was obtained from the same control subject.

1. *Study of* $^{131}I-T_4$

The amounts of $^{131}I-T_3$ representing the contamination in the $^{131}I-T_4$ sample were measured in solution I used for the estimation of T_4-binding capacity of the blood-protein fractions. Part of the solution was dropped onto the start line on Schleicher-Schüll filter paper No. 2043/B. The chromatogram was developed in N-butanol : dioxane : ammonia 2N (4 : 1 : 5). The strips were cut into 1 cm pieces and their activities were measured with an energy-selective counter type Gamma NK 108 in a highly collimated NaI- crystal detector. The imp/min values per Rf for T_4 (0.56) and for T_3 (0.63) were expressed in per cent of the total activity.

2. For the measurement of the T_4-binding capacity of the blood-protein fractions, the procedure described by LEMARCHAND-BÉRAUD et al. [4] was used with some modifications (REVICZKY et al. and SZÁNTÓ [7]). 0.1 ml of the test serum was incubated at 38°C with 0.1 ml of the $^{131}I-T_4$ solution I, and a further 0.1 ml aliquot of the serum with 0.1 ml of solution I/a. This was followed by electrophoresis, both mixtures being run in tris-maleinate buffer of 0.1 ionic for 16 hours, using a Zeiss–Jena apparatus (16 V, 16 mA, 3 V/cm). The temperature was kept at 24°C. The strips were autoradiographed and the imp/min values for the dark areas were measured with the same instrument against a third strip the treatment of which had been confined to staining with acid fuchsin. The imp/min values of the spots were expressed in per cents of the total activity. The shift in the radioactivity of the samples enriched with inactive thyroxine, versus the carrier-free samples is shown in Table I (Table I).

Table I

Name	Percentage distribution of radioactivity					
	Sol. I.			Sol. II/a.		
	TBG	TBA	TBPA	TBG	TBA	TBPA
L. R. A.	57.5	26.5	15.8	47.4	30.2	22.4

Calculation: L. R.A.
Concentration of Solution II

$$\text{PBI} = \frac{0.048\ \mu g/1\ ml}{0.800\ \mu g/1\ ml} = \frac{0.848\ \mu g/2\ ml}{0.424\ \mu g/ml}$$

As it can be seen from Table I, in the serum containing additional inactive T_4, TBG accounted for 47% of the total radioactivity. In the given case $x = \frac{47.4 \times 0.424}{100} = 0.20$. TBG is thus able to take up 0.20 μg/ml of T_4-iodine from 0.424 μg/ml PBI.

3. Purity test of the $^{131}I-T_3$-solution, i.e. measurement of the disintegration products formed along the front line of the chromatograms, from the sample diluted for the Hamolsky test, was done by the procedure employed for thyroxine.

4. The global T_3-binding capacity of blood proteins was measured according to HAMOLSKY (1957, 1959). 3 ml heparinized blood was incubated with 0.1 ml of the $^{131}I—T_3$ solution (0.4—0.8 μg/100 ml) then, after centrifugation and washing, the imp/min/ml values of the portion attached to the red blood cells were measured, expressed in per cents of the global imp/min value and corrected for 100 haematocrit. This value expressed the amount of T_3 which had not bound to blood proteins, thus giving indirect information on the saturation of blood proteins (TBP).

Results

The data of nine batches of $^{131}I—T_4$ are shown in Table II. In the first four columns we find the times of delivery, of processing, the percentage values of $^{131}I—T_4$ and of its disintegration product, $^{131}I—T_3$. The fifth and sixth columns represent the T_4-binding capacity of TBG and TBPA in term of T_4/100 serum, derived from the study of the other portion of the radioactive thyroxine solution. The seventh column gives the PBI values, the uniformity of the figures being indicative of the stability of thyroid function.

The samples contained variable amounts of T_4; the limits were 2.0 and 88.9%. While the T_4-binding capacity ranged from 7.1 to 27.4 μg/100 ml for TBG and from 1.0 to 18.0 μg/100 ml for TBPA, the PBI levels varied between 4.0 and 4.9 μg/100 ml. In the September batch, the T_4 content was in excess of 60% throughout the whole period of study, and the variations in T_4-binding capacity were not in excess of 19.3 to 22.3 μg/100 ml for TBG, and of 2.0 to 8.6 μg/100 ml for TBPA.

In order to check the findings shown in Table II, control tests were undertaken; their results are presented in Fig. 1. The two coordinate systems, one in the upper, one in the lower part of the diagram, correlate the values for the radioactive substance and for the bioassays. In the lower part of the left side we find the results of five tests performed on a $^{131}I—T_4$ sample obtained in 1971. The elongated lines in the upper section represent the amounts of TBG-bound T_4 in the serum of the same control subject. While this value declines parallel with the T_4 content, the TBPA-binding capacity (broken lines) increases parallel with the T_3 content of the samples. The data seen on the right side of the diagram represent the control values of this experiment. In this case, $^{131}I—T_3$ was used instead of $^{131}I—T_4$, in addition to the inactive T_4, for the saturation of blood serum. The broken lines in the lower system represent the T_3 content. The elongated lines in the upper section represent the binding capacity for TBG, the broken lines that for TBPA calculated from the activities bound to these fractions. Compared with the results of the $^{131}I—T_4$ assays, the TBG—T_3 binding capacity was considerably reduced, whereas that of TBPA, significantly increased, the values for $^{131}I—T_4$ having decreased from 19.5 μg/100 ml to 8.5 μg/100 ml for TBG, and increased from 11 μg/100 ml to 22 μg/100 ml for TBPA.

Table II

Stability of ^{131}I-thyroxine; modifications of the values as a result of deiodination, reflected in the thyroxine-binding capacity of TBG and TBPA

Day of		^{131}I–T_4	^{131}I–T_3	T_4 binding capacity, μg/100 ml serum		PBI μg/100 ml serum
Delivery	Assay	content, per cent		TBG	TBPA	
1970 II. 9.						
	II. 16	3.4	82.1	8.00	10.5	4.4
	II. 17	15.7	78.4	12.50	12.0	4.6
	II. 20	75.6	87.7	13.00	14.0	4.5
	II. 24	65.1	14.2	18.00	8.5	4.4
1970 III. 9.						
	III. 17	43.5	43.3	11.50	12.5	4.6
	III. 18	62.0	6.6	16.00	7.0	4.5
	III. 19	63.4	11.3	23.50	4.0	4.5
	III. 23	49.4	11.4	22.50	4.0	4.4
	III. 24	52.8	31.5	20.50	7.5	4.4
1970 IV. 14.						
	IV. 22	34.5	33.2	10.50	12.0	4.6
	IV. 24	75.0	8.5	14.50	3.0	4.5
	IV. 27	76.0	7.0	17.00	1.0	4.5
1970 VI. 1.						
	VI. 8	2.0	85.9	11.00	18.0	4.7
	VI. 9	14.5	75.0	14.50	13.0	4.5
	VI. 10	11.2	84.3	14.50	10.5	4.5
	VI. 11	12.5	80.4	13.50	12.0	4.6
1970 VI. 29.						
	VII. 14	5.4	73.0	9.50	11.0	4.7
	VII. 15	7.0	67.7	10.00	11.5	4.5
1970 VIII. 21.						
	IX. 3	17.7	70.0	9.50	11.5	4.8
	IX. 7	16.4	80.7	11.50	9.5	4.7
	IX. 8	20.1	73.2	12.70	13.0	4.6
1970 IX. 21.						
	IX. 22	88.9	5.6	19.80	8.6	4.3
	IX. 26	88.6	8.3	22.30	2.0	4.0
	IX. 29	80.5	10.2	20.30	4.5	4.3
	X. 1	84.3	6.3	21.30	3.5	4.2
	X. 5	64.9	10.1	19.30	5.0	4.0
1970 X. 20.						
	X. 22	21.8	71.3	16.20	10.0	4.1
	X. 24	24.3	63.3	18.20	11.2	4.3
	X. 28	23.5	64.0	14.70	7.6	4.4
	XI. 1	16.9	75.5	14.70	14.7	4.3
	XI. 3	81.6	7.7	27.40	3.0	4.1
1970 XI. 16.						
	XI. 18	17.3	67.8	14.20	12.20	4.1
	XI. 23	19.3	66.9	18.20	10.10	4.0
	XI. 25	20.5	67.9	11.10	15.70	4.1
	XI. 30	19.50	65.8	16.60	8.60	4.2
	XII. 2	11.9	69.1	7.10	17.80	4.0

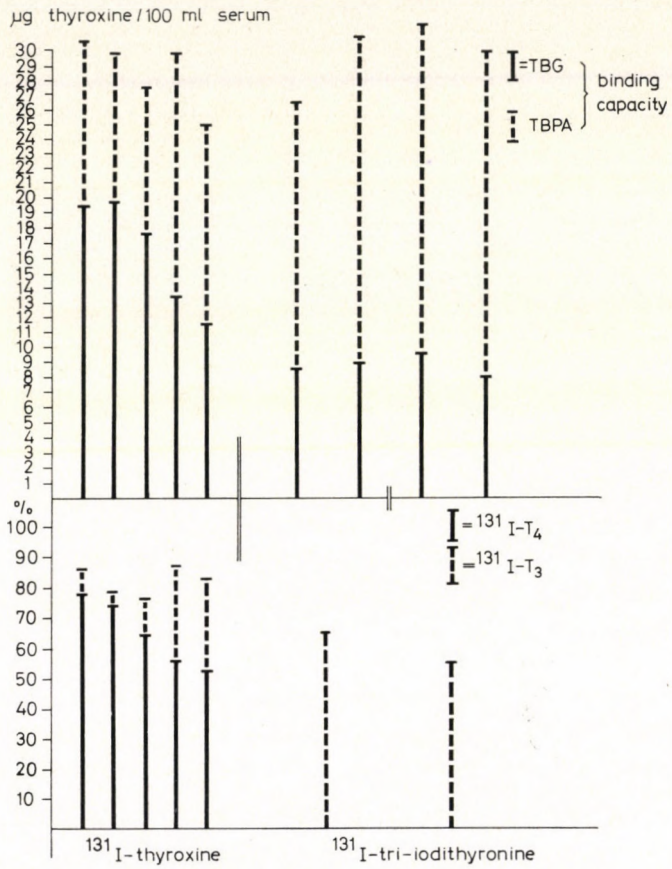

Fig. 1

The second series of studies was concerned with the stability, the preservability of $^{131}I—T_3$, and the influence of its changes on the tests with reference to the values of the Hamolsky test of the same control subject.

The first four columns of Table III contain the times of delivery, of assays, the T_3 content in per cent and the disintegration products demonstrable in the front line. The fifth column gives the results of the Hamolsky test in per cents, the sixth the PBI values. It is that the T_3 content, even at this high dilution, was mostly above 60%. The values of the Hamolsky tests were between 11 and 15%, which corresponds to the range of normal sera.

Discussion

1. As Table II shows, the $^{131}I—T_4$ solution undergoes some degree of disintegration before processing while still under guarantee, as reflected by the decrease in its T_4 content and the parallel increase in its T_3 content. This

Table III

Day of		$^{131}I-T_3$	$^{131}I-$Front	Hamolsky per cent Haematocrit = 100	PBI μg/100 ml serum
Delivery	Assay				
1970 II. 16.	II. 18	86.5	3.3	12	4.5
	II. 20	80.6	4.6	13	4.4
	II. 23	70.6	2.4	12	4.5
	III. 2	17.5	65.0	9	4.6
1970 III. 2.	III. 5.	70.2	12.8	15	4.7
	III. 10	52.5	19.4	12	4.5
	III. 16	61.4	21.6	11	4.4
	III. 19	50.0	9.0	10	4.4
1970 III. 31.					
	IV. 13	61.8	1.3	13	4.5
	IV. 14	56.7	1.5	11	4.4
1970 VI. 8	VI. 26	75.5	7.6	13	4.5
1970 IX. 7.	IX. 9	63.6	14.6	12	4.6
	IX. 14	59.1	6.8	11	4.5
	IX. 16	40.5	9.9	11	4.4
1970 IX. 21	IX. 22	70.1	12.1	12	4.6
	IX. 26	65.4	3.9	12	4.5
	IX. 29	62.0	3.0	12	4.4
	X. 2	64.7	2.1	12	4.7
	X. 6	62.0	5.0	11	4.5
1970 X. 20	X. 22	81.4	5.3	14	4.5
	X. 26	69.5	11.7	13	4.7
	X. 28	54.2	8.4	13	4.6
	XI. 4	53.6	11.5	10	4.7
1970 XI. 16	XI. 18	63.4	21.5	11	4.5
	XI. 23	73.6	3.4	12	4.6
	XI. 30	57.6	21.8	10	4.5

may have various explanations. Although, according to the catalogue, $^{131}I-T_4$ is being delivered within the first week after its preparation, there may be none the less some accidental protraction of prestorage in individual instances. $^{131}I-T_4$ is delivered in 50% propylene glycol solution. According to the measurements of the Research Institute of Organic Chemistry and Chemical Industry, the DK constant of the solution is 62.7. In an earlier study [12] we have shown that deiodination of T_4 increases in proportion to the rise in the

DK factor of the solvent. Then, $^{131}I—T_4$ being liable to disintegration, it may be affected by the variations of temperature during transportation as also by the process of dilution.

2. Columns 5, 6 and 7 of Table II show that with the decline of the T_4 content, the binding capacity of TBG tends to diminish, while that of TBPA increases, a phenomenon which may be ascribed to an artifact production, probably as a result of accumulation of T_3. The contaminations of the various batches of labelled thyroxine have been examined by several workers, and increased T_4 contents have been demonstrated even in the case of a minimum disintegration of $^{131}I—T_4$ (SCHUSSLER et al. [13]; VOLPERT et al. [14]; HERMANN et al. [15]; SELIGSON and SELIGSON [16]). The solution was found to be best suited for utilization 10 to 14 days after its preparation (HERMANN et al. [15]). $^{131}I—T_4$ was found to remain relatively stable for 20 days. Beyond the 30th day after its preparation, its disintegration rate increases rapidly (SELIGSON and SELIGSON [16]).

The isotope-dilution technique is of no practical use, unless the administered active substance and the inactive solvent are identical in qualitative respects. Since the inactive solution contained T_4 only, on the other hand, the amounts of T_3 contained in the radioactive solution increased parallel with the decrease in its T_4 content, the radioactivity measurements failed to provide information on the true binding conditions of T_4. As mentioned in the introduction of this study, T_4 attaches more strongly to TBG than does T_3 and is therefore capable of displacing it from this bond. We are thus justified in assuming that it is not T_4 but rather its disintegration product T_3 which accounts for the radioactivity measured in TBPA. This was supported by the results of the control tests presented in Fig. 1, where it can be seen that, while in the same serum the binding capacity of TBG declined sharply, that of TBGA increased to the same extent in the course of the utilization of labelled $^{131}I—T_3$.

Earlier authors (TATA [17]; BROWN-GRANT et al. [18]) do not refer to T_3-TBPA binding. It is on the evidence of the studies by DAVIS et al. [19], that the T_3-binding capacity of TBPA has been confirmed.

In view of the results of the present study, before seeking information on the thyroxine-binding capacity of the blood proteins, it should be realized that the figures obtained do not allow any definite diagnostic conclusion. This is illustrated by Fig. 2 presenting the values for hypothyroid, euthyroid and hyperthyroid subjects on the left side. The right side is confined to the limit values for T_4-binding capacity obtained over a one-year period in a normal serum. It can be seen that the fluctuations due to artifact production are in excess of the differences between the average values representing the individual states of thyroid function (Fig. 2).

The criteria for the accuracy of the bioassays in question may be formulated as follows.

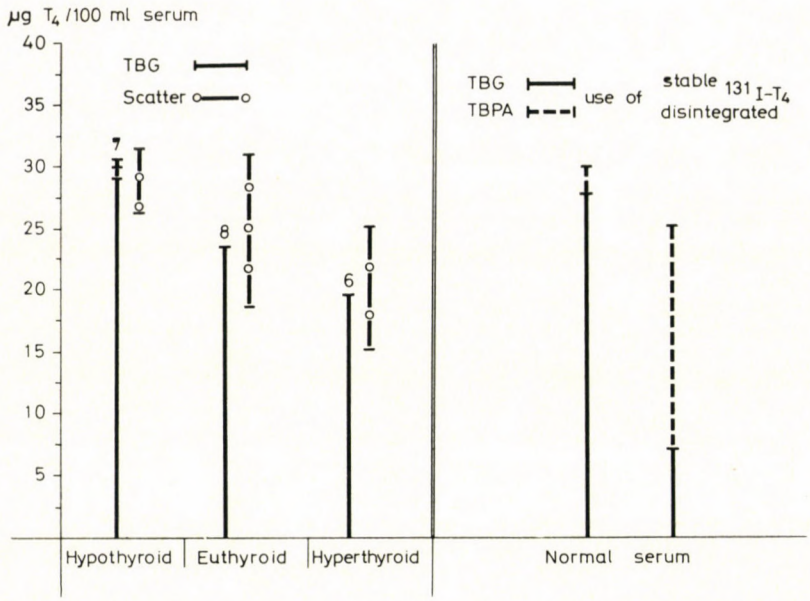

Fig. 2

— The suppliers should give explicitly the date of synthesis and the duration of prestorage, so as to prevent the use of the reagents beyond the 10th to 14th day after its preparation;

— the labelled hormones should be supplied in substance form or as solution of low DK;

— strict conditions of refrigeration are obligatory throughout all phases of transportation;

— since the unstable I-atom of T_4 at position 5' has given cause to various misinterpretations, it would be desirable to apply a tag to the non-detaching C-or H-atom too. The use of a double-tagged thyroxine might help to clarify numerous biological problems.

3. The amounts of T_3 contained in ^{131}I—T_3 are fairly constant, although the factors likely to affect it, i.e. protracted prestorage, relatively high DK of the 50% aqueous propylene-glycol solution, the harmful influence of transportation, etc. are the same as in the case of radioactive thyroxine. On the evidence of our earlier study (Reviczky and Nagy [12]) the DK factor of the medium has little influence of the release of iodine from T_3. Its relative stability makes it therefore suitable for assays which give fairly reliable values.

4. The scatter of the figures involved by the use of the relatively stable ^{131}I—T_3 is not significant and remains within the normal range all throughout. The reliability of the Hamolsky test is illustrated by the diagram in Fig. 3, representing the data for 10 patients in each group. There is a distinct demarca-

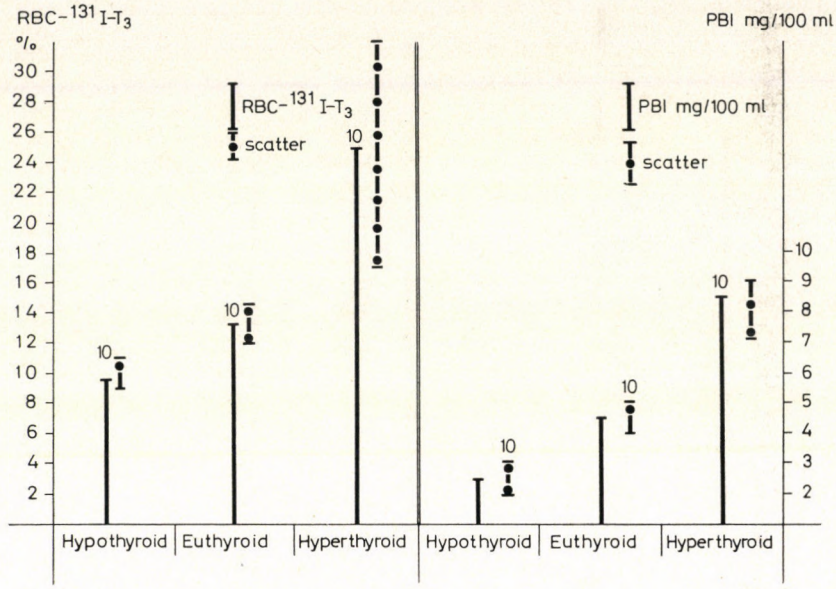

Fig. 3

tion between the zones of the hypothyroid, euthyroid and hyperthyroid values. We found no overlap either in these or in the corresponding PBI figures.

It has thus been confirmed that the Hamolsky test offers fairly reliable information on the function of the thyroid, and that it furnishes relevant diagnostic and therapeutic information. On the other hand, estimation of the thyroxine-binding capacity of the blood- protein fractions by the isotope-dilution technique gives uncertain results, therefore its value is highly questionable in both diagnostic and therapeutic respects.

Acknowledgements

The authors are indebted to Mrs. J. BARANYAI, Mrs. NAGY-PETRILLA and Mrs. I. TÓTH for technical assistance.

REFERENCES

1. FRIEDRICH, R., RIPPMANN, E. T., KAUFMANN, V.: Die Beeinflussung der Bindungsfähigkeit der thyroxinbindenden Proteine durch die Schwangerschaft. Schweiz. med. Wschr. **99,** 833—839 (1969).
2. WAHNER, H. W., WALSER, A. H.: Measurements of Thyroxine-Plasma Protein Interactions. Med. Clin. N. Amer. **56,** 849—859 (1972).
3. LARSEN, P. R.: Salicylate-induced Increases in Free Triiodothyronine in Human Serum. Evidence of Inhibition of Triiodothyronine Binding to Thyroxine-binding globulin and Thyroxine-binding Prealbumin. J. clin. Invest. **51,** 1125—1134 (1972).
4. LEMARCHAND-BÉRAUD, TH., ASSAYAH, M. R., VANNOTTI, A.: Alterations of Thyroxine-binding Protein in Clinically Hypo- and Hyperthyroid Patients with Normal PBI Level. Acta endocr. (Kbh.) **45,** 99—113 (1964).

5. HAMOLSKY, M. W., STEIN, M., FREEDBERG, A. S.: The thyroid hormone plasma protein complex in man. II. A new in vitro method for study of "uptake" of labelled hormonal components by human erythrocytes. J. clin. Endocr. **17,** 33 (1957).
6. HAMOLSKY, M. W., GOLODETZ, A., FREEDBERG, A. S.: The plasma protein thyroid hormone complex in man. III. Further studies on the use of the in vitro red blood cell uptake of I^{131}-l-triiodothyronine as a diagnostic test of thyroid function. J. clin. Endocr. **19,** 103—116 (1959).
7. REVICZKY, A. L., SZÁNTÓ, L.: Correlation between the thyroxine-binding capacity of blood proteins and the serum organic iodine level. Acta physiol. Acad. Sci. Hung. **32,** 337—348 (1967).
8. DAVIS, P. J., GREGERMAN, R. I.: Separation of Thyroxine-binding Proteins in Human Serum at pH 7.4. II. Effect of pH and Temperature on the Binding Capacities of Thyroxine-binding Globulin (TBG) and Thyroxine-binding Prealbumin (TBPA). J. clin. Endocr. **33,** 699—708 (1971).
9. OPPENHEIMER, J. H., MARTINEZ, M., BERNSTEIN, G.: Determination of the maximal binding capacity and protein concentration of thyroxine-binding prealbumin in human serum. J. Lab. clin. Med. **67,** 500—509 (1966).
10. TATA, J. R.: Transport of Thyroid hormones. Brit. med. Bull. **16,** 142—147 (1960).
11. REVICZKY, A. L., SZÁNTÓ, L., GRYNAEUS, T., MAGONY, I.: Deiodination of the ^{131}I-Labelled Substances of Blood Serum. I. Acta physiol. Acad. Sci. Hung. **29,** 107—120 (1966).
12. REVICZKY, A. L., NAGY, S. B.: Untersuchung der Thyroxindejodisation mit instrumenteller analytischer Methode. Endokrinologie **56,** 81—91 (1970).
13. SCHUSSLER, G. C., PLAPER, J. E.: Effect of Preliminary Purification of ^{131}I-Thyroxine on the Determination of Free Thyroxine in Serum. J. clin. Endocr. **27,** 242—250, (1967).
14. VOLPERT, E. M., MARTINEZ, M., OPPENHEIMER, J. H.: Radioiodinated Impurities in Commercial Preparations of ^{131}I-Thyroxine and Their Effect on the Measurement of Free Thyroxine in Human Serum by Equilibrium Dialysis. J. clin. Endocr. **27,** 421—428 (1967).
15. HERMANN, J., KRÜSKEMPER, H. L., MÜLLER, H.: Zur Methodik der Bestimmung von freiem, dialysablem Thyroxin in Serum. Clin. chim. Acta **24,** 457—466 (1969).
16. SELIGSON, H., SELIGSON, D.: Measurement of Thyroxine by Competive Protein Binding. Clin. chim. Acta **38,** 199—205 (1972).
17. TATA, J. R.: The effect of Self- and External Radiations on ^{131}I-Labelled L-Thyroxine and 3,5,3'-Triiodo-L-Thyronine in Solution. Clin. chim. Acta. **4,** 427—337 (1959).
18. BROWN-GRANT, K., BRENNAN, R. D., YATES, F. E.: Simulation of the Thyroid Hormone-binding Protein Interactions in Human Plasma. J. clin. Endocr. **30,** 733—751, (1970).
19. DAVIS, P. J., HANDWERGER, B. S., GREREMAN, R. I.: Thyroid Hormone Binding by Human Serum Prealbumin (TBPA). Electrophoretic Studies of Triiodothyronine-TBPA Interaction. J. clin. Invest. **51,** 515—521 (1972).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (1—2), pp. 131—137 (1974)

MECHANISM OF ACTION OF DI-IODOTYROSINE AND OF IODINE

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(Received February 7, 1974)

Model experiments for the clarification of the mechanism of di-iodotyrosine activity were undertaken in laboratory animals, and the results were compared with those obtained with KI. Neither of the substances was found inhibitory to the stimulative activity of the thyrotropin-releasing hormone on the release of TSH, and in acute experiments they did not affect the sensitivity of the thyroid to TSH and LATS. Di-iodotyrosine in large doses was found to slow down elimination of labelled thyroxine, whereas KI showed no activity of this kind.

It is uncertain whether di-iodotyrosine (DIT) has any effect on thyroid function. If it has, then it must be essentially a iodine-effect, since from the administration to humans not more than 8% is excreted with the urine unchanged, the rest leaving the body in the form of iodide [15, 18]. Iodine, on its part, acts as a suppressor of thyroid function, and the following mechanisms have been alleged to account for its suppressive effect.

1. Direct action on the thyroid;
2. suppression of thyroid sensitivity to TSH;
3. inhibition of pituitary TSH secretion;
4. modification of the peripheral metabolism of thyroid hormones.

Though the view that iodine acts directly on the thyroid has gained prevalence [3, 20] it does not seem justified to reject the other possibilities.

In order to gain insight into the mechanism of action of DIT, we have studied in mice and rats whether and to what extent it affected the sensitivity of the thyroid to TSH and LATS and whether it counteracted the TSH secretion-stimulating effect of the tyrotropin-releasing hormone (TRH). The effect of DIT on the peripheral metabolism of thyroxine has been also examined. The results have been compared with those obtained with KI.

Material and method

Thyroid responsiveness to TSH and to LATS was studied by our earlier procedure based on the McKenzie test, in albino mice weighing approximately 20 g [6]. Groups of 10 mice were formed. The animals were injected subcutaneously with 6 μCi ^{131}I, this was followed by the administration of 4 μg l-tri-iodothyronine on one occasion and of 2 μg, on two occasions. The actual assays were carried out on the 4th day after administration of the isotope, the TSH- and LATS-containing test substances being administered intravenously. A control group

received 5% cent human serum albumin (HSA) intravenously in 0.5 ml doses. Blood was withdrawn from the animals of the individual groups prior to and at 2 and 8 hours after administration of the substances. The samples were measured for radioactivity in a well-type scintillation detector at the photopeak of ^{131}I. The mean activities of the TSH- and LATS-treated groups were referred to the mean activities obtained in the HSA-treated control group (control = = 100%) and expressed in terms of per cent (TSH- or LATS-index per cent). For TSH, the 2-hour figures, for LTAS the 8-hour figures, were considered.

Statistical analysis was done with log-metameters. The values for the individual groups referred to those of the HSA-treated control group were computed from the geometrical means. In the Tables, the 95% fiducial limits are presented below the mean values. When there was no overlap between the 95% fiducial limits, then the deviations, at least at a 5% level, were considered mathematically significant, i.e. $P < 5\%$. When the fiducial limits of the two mean values were found to overlap or to be close to each other, then the differences were not regarded as significant. On the other hand, the effect was considered significant in the case of wide differences between the fiducial limits.

For the study of responsiveness to TSH, the animals were injected intravenously with 0.80 mU lyophilized TSH (Ambinon, Organon) or with 0.015 international standard U LATS. In the first and second series of studies, the animals received 1 mg KI or 2 mg DIT, respectively, by the intravenous route, simultaneously with the hormones. In the third series they were injected intraperitoneally with the above doses of KI and DIT, respectively, 30 min before administration of the hormones.

In the further course of the studies it was examined whether DIT and KI were able to counteract the TRH-induced enhancement of TSH secretion. For this purpose, albino rats of 200 g body weight were injected with 200 and 400 ng/100 g TRH (Hoechst A. G.) by the intravenous route, against a control group injected with 0.5 ml doses of 5% HSA. Twenty min later blood was withdrawn and tested for TSH by the modified McKenzie test, in groups of 10 mice. Part of the rats received DIT, KI and tri-iodothyronine (T_3) in different doses subcutaneously 2 hours before the administration of TRH (Tables III and IV).

Finally, it was examined in groups of 10 mice each whether and to what extent DIT and iodine affected the clearance of labelled thyroxine (T_4*). In accordance with the McKenzie test, the animals were given T_3 for the suppression of endogenous TSH secretion and injected on the 3rd day of pretreatment with 1.0 μCi ^{125}I-thyroxine (Amersham). 24 hours later the animals were injected intravenously with different doses of KI, DIT, thyroxine and thyroxine + DIT, respectively. Blood was withdrawn from the individual animal groups prior to and 8 hours after administration of the test substances, and its activity was measured in a well-type scintillation detector. The decline of activity reflected the elimination of T_4.

Results

TSH and LATS were found to enhance the release of hormonal iodine from the thyroid. It can be seen in Tables I and II that iodine or DIT, administered in large doses, failed to affect the thyroid-stimulating effect of TSH and LATS. (The mean values for the groups having received additional KI or DIT fell within the fiducial limits of the groups to which exclusively TSH or LATS had been administered; the differences were thus non-significant.) In groups I and II, iodine or DIT was administered intravenously together with TSH or LATS. On the other hand, in group III, the administration of iodine and of DIT preceded by 30 min the intravenous administration of the thyroid-stimulating hormones.

In Table III it is shown that T_3 in doses exceeding 5 μg has a depressive effect on the TSH response to TRH. The differences in the responses to TRH between the animal groups pretreated with 5 μg doses of T_3 on the one hand and the untreated groups on the other, were significant both biologically and mathematically, and T_3 pretreatment with 50 μg doses resulted in a complete

Table I

Thyroid sensitivity to TSH—LATS. Effect of KI and DIT on hormone release from the thyroid in the acute experiment. KI and DIT were administered intravenously together with TSH and LATS, respectively. (The 95% fiducial limits in brackets)

Series	Group	KI	DIT	Substance administered	Dose	TSH index per cent	LATS index per cent
I	1	—	—	TSH	0.8 mU	657 (618—696)	—
	2	1 mg	—	TSH	0.8 mU	621 (599—643)	—
	3	—	2 mg	TSH	0.8 mU	663 (629—697)	—
	4	—	—	LATS	0.015 U	—	690 (663—717)
	5	1 mg	—	LATS	0.015 U	—	656 (628—684)
	6	—	2 mg	LATS	0.015 U	—	671 (636—706)
II	1	—	—	TSH	0.8 mU	623 (592—654)	
	2	1 mg	—	TSH	0.8 mU	637 (605—669)	
	3	—	2 mg	TSH	0.8 mU	618 (596—640)	
	4	—	—	LATS	0.015 U	—	721 (682—760)
	5	1 mg	—	LATS	0.015 U	—	697 (664—730)
	6	—	2 mg	LATS	0.015 U	—	712 (674—750)

Table II

Thyroid sensitivity to TSH—LATS. Effect of KI and DIT on hormone release from the thyroid in the acute experiment. KI and DIT were administered intraperitoneally 30 min before the intravenous injection of TSH and LATS, respectively. (The 95% fiducial limits in brackets)

Series	Group	KI	DIT	Administered substance	Dose	TSH index per cent	LATS index per cent
III	1	—	—	TSH	0.8 mU	636 (609—663)	
	2	1 mg	—	TSH	0.8 mU	647 (617—677)	
	3	—	2 mg	TSH	0.8 mU	620 (598—642)	
	4	—	—	LATS	0.015 U		653 (628—678)
	5	1 mg	—	LATS	0.015 U		644 (613—675)
	6	—	2 mg	LATS	0.015 U		637 (616—658)

suppression of the TRH-induced increase in the release of thyrotropin. On the other hand, as it can be seen in Table IV, even excessive doses (1000 μg of KI and of DIT) failed to inhibit the stimulating effect of TRH on the release of TSH.

Finally, Table V demonstrates that the rate of T_4* elimination was not affected by KI. Intravenous administration of DIT in large doses caused

Table III

Effect of triiodothyronine pretreatment on the TRH increased release of TSH

Series	Controls	TRH	TRH + T_3 (0.5 μg)	TRH + T_3 (5 μg)	TRH + T_3 (25 μg)	TRH + T_3 (50 μg)
I	100 (90—110)	237* (217—257)	242 (224—261)	178 (170—185)	—	101 (83—118)
II	145 (140—150)	429 (396—463)	435 (379—491)	360 (350—370)	204 (200—208)	—
III	262 (256—268)	561 (554—568)	—	482 (477—487)	385 (375—392)	265 (261—270)

a. —* indicates the group in which the dose of TRH was 200 ng/100 g instead of the 400 ng/100 g administered to all other groups.
b. — the mice in the control group received 0.5 ml of the serum of TRH-untreated rats.
c. — the 95% fiducial limits in brackets.

Table IV

Effect of DIT and KI pretreatment on the TRH increased release of TSH

Series	Controls	TRH	TRH + DIT 50 μg	TRH + DIT 100 μg	TRH + DIT 1000 μg
IV	251 (243—258)	378* (365—391)	374 (369—379)	369 (361—378)	389 (376—402)
V	136 (122—151)	468 (411—525)	494 (474—514)	488 (464—513)	500 (476—523)
VI	215 (204—226)	685 (668—702)	682 (660—695)	685 (669—701)	682 (663—696)
	Controls	TRH	TRH + KJ 50 μg	TRH + KJ 100 μg	TRH + KJ 1000 μg
VII	104 (98—110)	286* (276—295)	281 (275—287)	277 (266—287)	281 (272—290)
VIII	106 (101—112)	481 (296—566)	513 (495—532)	500 (490—510)	512 (492—531)

Notes:

a. —* indicates the group in which the dose of TRH was 200 ng/100 g instead of the 400 ng/100 g administered to all other groups.
b. — the mice in the control group received 0.5 ml of the serum of TRH-untreated rats.
c. — the 95% fiducial limits in brackets.

some delay in the disappearance of T_4* from the circulation, as reflected by the 8-hour activity values in the DIT-treated animals, which were higher than in the controls. Yet, even massive doses of DIT failed to counteract the enhancing effect of the large thyroxine doses on the elimination of T_4*.

Table V

Effect of DIT, KI and thyroxine on the elimination of labelled thyroxine in mice.

Series	Substance administered	Dose	Percentage of initial value	Fiducial limits
I	Control	—	57.1	55.0—59.1
	DIT	100 μg	57.0	56.5—57.4
	DIT	500 μg	79.8	77.4—32.1
	DIT	1000 μg	79.8	78.9—80.8
	KJ	500 μg	57.4	55.8—58.9
II	Control	—	60.9	59.3—62.7
	DIT	100 μg	60.1	50.9—63.3
	DIT	500 μg	75.3	72.9—77.8
	DIT	1000 μg	82.7	80.3—85.2
IV	Control	—	52.0	49.3—54.8
	T_4	50 μg	37.2	32.4—39.0
	T_4 + DIT	50 μg 1000 μg	37.2	32.6—38.2
	Control	—	49.6	47.9—51.3
	T_4	50 μg	38.2	36.9—39.7
	T_4 DIT	50 μg 1000 μg	36.6	35.6—37.6

Note:
The animals in the control received 5% human serum albumin.

Discussion

Iodine is known to exert its thyroid function decreasing effect by the interaction of TSH. It has therefore been assumed that iodine inhibited pituitary TSH secretion [5, 14]. These findings have, however, not been confirmed by recent evidence [1] and even the intrapituitary injection of iodine has failed to slow down the hormone release from the thyroid [10]. Clinical observations also suggest that the effect of iodine on the thyroid is unrelated to TSH secretion [2, 9, 20]. This is consistent with the results of our earlier studies, according to which iodine fails to suppress the enhancing effect of methyl-mercaptoimidazol on the release of TSH, and also to reduce the high serum TSH level in primary hypothyroidism [7]. DIT has been less extensively studied from this aspect. The findings of VON ZUR MÜHLEN et al. [19] indicate that DIT has no inhibitory effect on TSH secretion in primary hypothyroidism.

Administration of TRH is known to enhance the release of TSH from the pituitary and to raise the serum TSH level [12]. It remains, however, to be clarified whether this enhanced TSH secretion was inhibited by DIT or iodine. According to our findings and in agreement with earlier observations [17], T_3 in doses exceeding 5 μg decreases the response to TRH, while even large doses of DIT or of KI do not interfere with the stimulatory effect of TRH on the release of TSH. According to our earlier as well as to the present studies, the enhanced release of TSH from the pituitary, whether due to stimulation from higher centres (TRH) or to low serum-thyroxine concentrations as a result of thyroid hypofunction, remains unaffected by KI and DIT alike.

The possibility has, however, to be considered that iodine might affect the sensitivity to TSH of the thyroid [11], and this has prompted us to examine the influence of iodine and of DIT on the thyroid response to TSH. The studies have then been extended to LATS also.

Iodine pretreatment is known to reduce radioiodine uptake by the thyroid. Therefore, in the present series of experiments, DIT and iodine were given together with or just before the administration of TSH and LATS. Under these conditions neither DIT nor iodine were found to affect the response to TSH and LATS of the thyroid. Our results support the earlier findings of Ochi and DeGroot [16] and have confirmed that even large doses of DIT have no influence on the effect on the thyroid of TSH and LATS.

The prompt response to iodine in hyperthyroidism was attributed earlier to an influence on the peripheral metabolism of thyroid hormones. Studies in animals and observations in humans have, however, failed to support this claim [4, 8]. On the evidence of the present study, the disappearance rate of T_4* is unaffected by KI and was slowed down by large intravenous doses of DIT. A possible explanation of this effect of DIT is that its metabolism probably involves the same peroxidase-enzyme system which takes part in the deiodination of T_4, therefore a competition for the enzyme may occur between T_4 and DIT at the periphery, thus allowing DIT to displace T_4 to some extent and to interfere with its deiodination. The inhibitory effect of thyroxine-analogues on the deiodination of T_4 has been attributed to a similar mechanism [13]. The present results are valid under the experimental conditions referred to above. Yet, it is questionable how far the effect of DIT is involved in thyroid hyperfunction, since in our mouse experiments even massive doses of DIT failed to counteract the stimulatory effect of thyroxine on the elimination of T_4*.

Acknowledgement

The thyrotropin-releasing hormone was kindly supplied by Farbwerke Hoechst A. G., Frankfurt a. M.; the LATS standard, by the Division of Biological Standards, National Institute for Medical Research, London, England. Their help is gratefully acknowledged.

REFERENCES

1. Abbassi, V., McKenzie, J. M.: Endocrinology **81,** 871 (1967).
2. Benus, R. S., Lippsett, M. B.: J. clin. Endocr. **19,** 19 (1959).
3. Burke, G.: J. clin. Endocr. **30,** 76 (1970).
4. DeGroot, L.: J. clin. Endocr. **26,** 778 (1966).
5. Del Conte, E., Stux, M.: Acta endocr. (Kbh.) **20,** 246 (1955).
6. Földes, J., Krasznai, I., Gyertyánfy, G., Piroska, E.: Orv. Hetil. **106,** 886 (1965).
7. Földes, J., Krasznai, I., Piroska, E., Gesztesi, E., Takács, I.: Endokrinologie **52,** 80 (1967).
8. Galton, V. A., Ingbar, S. H.: Endocrinology **81,** 1439 (1967).
9. Green, W. L., Ingbar, S. H.: J. clin. Invest. **41,** 173 (1962).
10. Gree, M. A., Yamada, T., Iino, S.: Ann. N. Y. Acad. Sci. **86,** 667 (1960).
11. Greer, M. A., DeGroot, L. J.: Metabolism **5,** 682 (1956).
12. Gual, C., Kastin, A., Schally, A. V.: Rec. Progr. Hormone Res. **28,** 173 (1972).
13. Larson, F. C., Albright, E. C.: J. clin. Invest. **40,** 1132 (1961).
14. Loeser, A.: Klin. Wschr. **13,** 533 (1934).
15. McGirr, E. M.: Brit. med. Bull. **16,** 113 (1960).
16. Ochi, Y., DeGroot, L. J.: Endocrinology **84,** 1305 (1969).
17. Schally, A.: Rec. Progr. Hormone Res. 510 (1968).
18. Stanbury, J. B., Meijer, J. W., Kassenaar, A. A.: J. clin. Endocr. **16,** 848 (1956).
19. Von z. Mühlen, A., Emrich, D., Hesch, R. D., Köbberling, J.: Acta endocr. (Kbh.) **68,** 669 (1971).
20. Wolff, J.: Amer. J. Med. **47,** 101 (1969).

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Printed in Hungary

A kiadásért felel az Akadémiai Kiadó igazgatója. Műszaki szerkesztő: Zacsik Annamária

A kézirat nyomdába érkezett: 1974. X. 16. — Terjedelem: 12,6 (A/5) ív, 58 ábra

75.1004 Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György

ACTA MEDICA

ТОМ 31 — ВЫП. 1—2

РЕЗЮМЕ

РОЛЬ СПИННОМОЗГОВЫХ МЕХАНИЗМОВ В РАЗВИТИИ ОСТРОГО ПОРАЖЕНИЯ ПЕЧЕНИ, ВЫЗВАННОГО ЧЕТЫРЕХХЛОРИСТЫМ УГЛЕРОДОМ

И. СЛАМКА, Й. МЕНЬХАРТ, Й. ШОМОДИ

Под влиянием перерезки спинного мозга, проведенной на крысах за два часа перед затравкой, характерное для четыреххлористого углерода поражающее печень действие значительно видоизменяется. Изменение целостности лизосом и гератоцеллюлярный некроз не развиваются, Ожирение печени возникает в более умеренной степени, содержание свободных жирных кислот в плазме не возрастает. После перерезки шейной части спинного мозга не наблюдается и ожирения печени. Если поражающий печень агент применяется через 4 дня после перерезки торакального отрезка спинного мозга, то патологический процесс в печени развивается. Согласно результатам опытов авторов, понижение температуры тела и факторы всасывания и транспорта не ответственны за наблюдаемые изменения. Можно сделать вывод, что спинномозговые центры играют пермиссивную роль в деле возникновения поражения печени.

ОБРАЗОВАНИЕ ПРОТИВОТЕЛ ПРОТИВ ЧЕЛОВЕЧЕСКОЙ КАТАРАКТЫ В ОПЫТАХ НА ЖИВОТНЫХ

Е. КИНЧЕШ и Ж. ЧАБА

Авторы проводили иммунизацию морских свинок и кроликов человеческими помутневшими хрусталиками. Половину животных иммунизировали вытяжкой, содержащей капсулу хрусталика, а другую половину вытяжкой без капсулы. У кроликов, иммунизированных безкапсульным гомогенизатом методом иммунэлектрофореза, было получено пять линий преципитации, а против хрусталика с капсулой — только 2 линии преципитации. У морских свинок не удалось выявить образования противотел против помутневшего хрусталика с капсулой, в то время как при применении вытяжки хрусталика без капсулы была получена одна линия преципитации.

Авторы полагают, что капсула хрусталика играет известную — пока еще невыясненную — роль в иммунных процессах.

ОПРЕДЕЛЕНИЕ ПРОСТРАНСТВЕННОЙ СКОРОСТИ ВЕКТОРНОЙ КАРДИОГРАММЫ ПРИ НАРУШЕНИЯХ ВНУТРИЖЕЛУДОЧКОВОГО ПРОВЕДЕНИЯ ИМПУЛЬСОВ

П. КЕНЕДИ, Д. МЮЛЛЕР и А. СЕКЕЙ

Из данных отведения x, y, z по Франку авторы определяли при помощи специальной аналоговой-ЭВМ пространственную скорость векторной кардиограммы и изобразили результат на самопишущем приборе в форме шкапярной кривой.

Они проводили анализ формы и количественных данных кривых. Были регистрированы кривые скорости 30 здоровых лиц. В 28 случаях блокады левой ножки Тавары замедление проведения наблюдалось на среднем участке комплекса QRS. Кривая пространственной скорости предоставляет возможность для обособления неосложненных и сочетанных инфарктом случаев блокады левой ножки. В 19 случаях блокады правой ножки авторы нашли значительное снижение скорости проведения во второй половине комплекса QRS. При определении кривой скорости в 17 случаях левой передней полублокады авторы нашли два хорошо обособляемых типа. У больных с электростимулятором правого и левого желудочков (11 случаев) после спайка большой скорости в соответствии с ретроградным распространением возбуждения наблюдается значительное замедление проведения.

На основе результатов исследований авторы устанавливают, что при нарушениях внутрижелудочковой проводимости кривая пространственной скорости предоставляет наиболее точную информацию о скорости проведения и о месте и размере замедления.

ПОМРАЧЕНИЯ СОЗНАНИЯ ПРИ МАЛОМ ПРИСТУПЕ ВО ВРЕМЯ НОЧНОГО СНА С ОСОБЫМ ВНИМАНИЕМ НА ПЕРЕХОДНЫЙ СОН И НА ПЕРИОДЫ БЫСТРОГО ДВИЖЕНИЯ ГЛАЗ (REM)

П. ХАЛАС и Е. ДЕВЕНЬИ

Авторы анализировали 12 полиграфических пластинок, взятых всю ночь у 11 больных, страдающих малыми приступами. Припадки малого приступа появлялись в период перехода от состояния бодрствования в период сна, во время преходящего пробуждения и в периоды перехода до и после сна. Подробный анализ переходных периодов — значит исследования динамизма между четырмя уровнями бодрствования в каждые 20 сек.— показал, что при малом приступе припадки помрачения сознания появляются после флюктуации уровня бодрствования и что существует оптимальная зона легко подавленного бодрствования, прохождение которой, в частности в направлении от более поверхностного к более глубокому сну, способствует появлению припадков помрачения сознания.

ОПЫТ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ЛИПОИДОВ В ПРОБАХ ПЕЧЕНОЧНОЙ ТКАНИ

Х. ДОНХОФФЕР

Автор проводил в пробах печени из материала 107 вскрытий в кусочках ткани весом в нескольких граммов гравиметрическое определение общего содержания липоидов. С другой стороны он определил в вытяжках 10—20 мг-овых проб тех же органов величины содержания холестерина, триглицаридов и фосфорлипидов. Величины общего содержания липоидов, полученные в макропробах, хорошо совпадали с суммой величин липоидных фракций, полученных в микропробах. В норме содержание жира в печени, предположительно, составляет 5,5% влажного веса. Более высокое содержание жира является признаком ожирения печени. Исследованиями автора подтверждается установление, согласно которому при жировом перерождении накопление триглицеридов не сопровождается увеличением фракций холестерина и фосфорлипида.

Описанная техника применима для анализа проб ткани, взятых in vivo при помощи иглы Менгини.

ИСПОЛЬЗОВАНИЕ ПОСТОЯННЫХ ЙОДНОЙ КИНЕТИКИДЛЯ ХАРАКТЕРИСТИКИ ФАРМАКОЛОГИЧЕСКИХ ЭФФЕКТОВ

К. Г. БАРТА

Автор применял для анализа действия лекарств, оказывающих непосредственное влияние на функцию щитовидной железы, трехячеечную модель. Он определил в опытах на животных постоянные йодной кинетики, фигурирующие в математических моделях и дифференциальных уравнениях, описывающих процесс, в качестве количеств, характерных для эффекта данного лекарства. В своих экспериментах автор изучала действие амбинона (ТСГ), тироксина и трийодтиронина, повышающих йодный обмен, а также действие метотирина, дийодтирозина и перхлората калия, понижающих йодный обмен.

Согласно наблюдениям влияние амбинона на йодный обмен организма соответствует литературным данным. Действие тироксина было меньше ожидаемого, а трийодтиронин оказал противоположное действие. Автор объясняет это наблюдение отчасти механизмом отрицательной обратной связи (feed back), и отчасти тем обстоятельством, что действующие вещества, в веденные в организм экзогенным путем, не проходят через все станции йодного оборота и, следовательно, если их точка приложения действие находится в опущенных фазах, то они — попадая в кровь — оказываются безэффективными.

В случае всех трех препаратов действие тиреостатических средств было однозначным и достоверным. Однако степень их эффекта зависит от механизма действия действующего начала и от способа его ведения.

ИЗУЧЕНИЕ ФУНКЦИИ ПОЧЕК И МОРФОЛОГИЧЕСКИХ ИЗМЕНЕНИЙ ПРИ ГИПЕРТОНИИ, ВЫЗВАННОЙ У КРЫС ОДНОСТОРОННИМ ЛИГИРОВАНИЕМ ПОЧЕЧНОЙ АРТЕРИИ

А. ФЕКЕТЕ, Е. ТАРЬЯН и Е. КОНЬЯР

Авторы вызывали у крыс гипертонию путем одностороннего лигирования почечной артерии при сохранении невредимой почки и изучали функцию почек и морфологические изменения. Было установлено, что на 13-й или 30-й неделе, рассматриваемых в отношении кровяного давления и гипертрофии органа как установившееся состояние (steady state), в отдельных параметрах функции почек наблюдаются отклонения по сравнению с контрольной группой. Одновременно с этим в невредимой почке были найдены поражения сосудов, характерные для гипертонии. Обсуждается значение гипертрофии с точки зрения нормальной функции и нормотонии и значение дегенерации сосудов с точки зрения гипертонии и дисфункции почек.

ИЗМЕНЕНИЯ КОНЦЕНТРАЦИИ ГЛИКОПРОТЕИДОВ В СЫВОРОТКЕ ПРИ МИОКАРДИАЛЬНОМ ИНФАРКТЕ

Л. ЯКОБ, Й. ФЕХЕР, И. ШИРО, Э. СОНДИ и Й. СЕКЕЙ

Методом радиальной иммунной диффузии была определена сывороточная концентрация IgG, IgA, IgM, церулоплазмина, α_2 макроглобулина и трансферрина при остром миокардиальном инфаркте. Авторы в течение 40 дней следили за изменениями и установили, что концентрация IgG и IgA повысилась от 16-го до 21-го дня. Наиболее выраженным было повышение концентрации церулоплазмина, которое даже по истечении 40 дней не прекратилось полностью. Подобная тенденция наблюдается в уровне α_2-макроглобулина, однако его изменения менее выраженные. Концентрация трансферрина понизилась. Обсуждается значение определения сывороточных гликопротеидов.

ИЗУЧЕНИЕ ЛЕЙКОТАКТИЧЕСКОГО ЭФФЕКТА ЧУЖЕРОДНОГО МОЛОКА В КОЖЕ КРОЛИКА

Ш. ТОТ, Г. КРАСНАИ и Т. СИЛАДЬИ

Полученное у здоровых женщин, свеже сцеженное молоко, и коровье молоко с 2,8%-ым содержанием жира при внутрикожном впрыскивании в кожу кроликов оказывают выраженное лейкотактическое действие, и поэтому этот метод применим для подготовки местной реакции Шварцмана. После инъекции возникает дерматит и в подкожной клетчатке наблюдается выраженная лейкоцитарная инфильтрация.

В день после подготовки на месте подготовки внутривенным впрыскиванием эндотоксина или 5—8 мл молока можно провоцировать тромбогеморрагическую реакцию, во многих отношениях напоминающую явление Шварцмана.

В подготовке реакции имеет значение сильное лейкотактическое действие молока.

Обсуждается возможное патологическое и практическое значение проводившихся авторами опытов на животных в области клиники.

ДАННЫЕ К ИЗМЕНЕНИЯМ ЭТЕРИФИЦИРОВАННЫХ КИСЛОТ ЖИРНОГО РЯДА — ТРИГЛИЦЕРИДОВ И ХОЛЕСТЕРОЛОВОГО ЭФИРА — В ПЛАЗМЕ КРОВИ ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ ГИПЕРТОНИИ КРЫСЫ

М. Л. МИХАЙЛОВ

Этерифицированные кислоты жирного ряда — триглицериды и холестероловый эфир — были изучены в плазме крови при экспериментальной гипертонии крысы. Наблюдалось достоверное повышение ненасыщенных этерифицированных кислот жирого ряда (ТГ), происходившие параллельно соответствующим изменениям, наблюдаемым в крови в спектре свободных, неэтерифицированных жирных кислот. При гипертонической нагрузке господствующее место насыщенного ряда занимает пальмитиновая кислота, а господствующее место ненасыщенного ряда занимает олеиновая кислота. Диаметрально противоположное поведение показывают при гипертонии этерифицированные жирные кислоты (холестероловый эфир). В этом случае наблюдается уменьшение насыщенных и соответствующее повышение ненасыщенных жирных кислот. Повышается также количество биологически важных эссенциальных жирных кислот, линолевой и арахидоновой кислот.

Обсуждаются некоторые аспекты жирного обмена в связи с повышенными энергетическими и функциональными потребностями при гипертонии. Изменения этерифицированных кислот жирного ряда следует рассматривать как адаптативный механизм.

ПРОТЕОЛИТИЧЕСКАЯ АКТИВНОСТЬ ЧЕЛОВЕЧЕСКОГО ЖЕЛУДОЧНОГО СОКА

Й. БАДУРСКИ, К. ЗВИЕРЗ и Б. БОГДАНИКОВА

Авторами была изучена протеолитическая активность человеческого желудочного сока. В качестве субстрата они применяли бычий альбумин и казеин. У 73% исследовавшихся лиц желудочный сок лучше переваривал казеин, а у 27% — альбумин. Эти результаты указывают на то, что для оценки протеолитической активности человеческого желудочного сока необходимо применять оба субстрата.

ПЛАЗМОЦИТАРНЫЙ МИОЗИТ ПРИ РЕВМАТОИДНОМ АРТРИТЕ

Е. МАДЬЯР, А. ТАЛЕРМАН, М. ФЕХЕР и Х. В. ВАУТЕРС

У 46-летнего мужчины с трехмесячной давностью ревматоидного артрита была произведена синовектомия правого запястья.

В материале мышечной биопсии, взятом в этот период, были выявлены обширные плазмоцитарные инфильтраты.

Аномалий иммуноглобулинов не было.

При произведении мышечной биопсии спустя 10 месяцев после операции, больше не удалось выявить плазмоклеточного инфильтрата.

Полагается, что плазмоклеточные инфильтраты, наблюдаемые в мышцах, являются ранней манифестацией ревматоидного заболевания.

КЛИНИЧЕСКИЕ ПАРАМЕТРЫ ДЛЯ ДИФФЕРЕНЦИАЛЬНОЙ ДИАГНОСТИКИ И ОПЕРАЦИОННОЙ ИНДИКАЦИИ ХОЛОДНЫХ УЗЛОВ ЩИТОВИДНОЙ ЖЕЛЕЗЫ

Д. БАЛАЖ, Ш. ФАЗЕКАШ, Л. СКОРСКИ, Д. ХАЙЕР, Г. ЧАКИ и М. СЕЛЕЦКИ

При анализе интра- и постоперационных гистологических диагнозов 300 случаев холодных узлов щитовидной железы из зобноэндемической равнинной территории авторы изучали частоту встречаемости рака щитовидной железы, потенциально злокачественных опухолей и тиреоидитов. Они выделяли абсолютные и относительные клинические параметры, оказывающие помощь при дифференциальной диагностике холодных узлов щитовидной железы, а также при определении правильного срока операции.

МОДЕЛЬНЫЙ МИОКАРДИТ У КРЫСЫ

II. Исследования при нагрузке

П. ШВАРЦМАНН, Й. ДЕМЕТЕР и Е. МАДЬЯР

Авторы вызвали у взрослой крысы диетой, содержащей этиловый эфир лауриновой кислоты, изменение, напоминающее интерстициальный миокардит у человека. При физической нагрузке животных изменения были более тяжелыми. Данные ЭКГ животных были сопоставлены с гистологической картиной. Устанавливается, что ЭКГ-изменения хорошо отражают протекание болезни.

ЛИМФОЦИТАРНЫЕ ПОВЕРХНОСТНЫЕ ИММУНГЛОБУЛИНЫ ПРИ АВТОИММУННЫХ ЗАБОЛЕВАНИЯХ

II. Лимфоцитотоксины на поверхности лимфоцитов

П. ГЕРГЕЙ, Д. СЕГЕДИ, Э. ШТЕНСКИ, Б. ФЕКЕТЕ, Г. САБО и Д. ПЕТРАНЬИ

Авторы изучали действие лимфоцитотоксинов SLE на лимфоциты, носящие поверхностные иммунглобулины. Методом косвенной иммунофлюоресценции можно выявить связывание лимфоцитотоксинов здоровыми лимфоцитами. Предварительное применение антигуманного иммунглобулина не препятствует связыванию лимфоцитотоксинов, значит, они связываются также Т-лимфоцитами. Под влиянием трипсина связанные лимфоцитотоксины освобождаются. У больных активным автоиммунным заболеванием в 20% случаев удалось выявить связанные лимфоцитотоксины. Обсуждается связь между клетками, носящими поверхностные иммунглобулины, и Б-лимфоцитами.

ИЗУЧЕНИЕ УСТОЙЧИВОСТИ ^{131}J-ТИРОЗИНА И ^{131}J-ТРИЙОДТИРОНИНА И ВЛИЯНИЕ РАДИОЛИТИЧЕСКОГО РАСПАДА НА ОТДЕЛЬНЫЕ ДИАГНОСТИЧЕСКИЕ ТЕСТЫ

А. Л. РЕВИЦКИ и Л. САНТО

В сыворотке крови одного и того же контрольного лица авторы изучали в течение года методом, основывающимся на принципе разведения изотопа, способность отдельных фракций белков крови (T*BG*, TBPA, TBA), транспортирующих гормоны щитовидной железы, к поглощению тироксина (T_4). В предписанном разведении примененного к насыщению $^{131}J—T_4$ (**Amersham RCC**) во всех случаях определяли соотношение $^{131}J—T_4$: $^{131}J—T_3$. Наблюдалась тенденция, что в случае накопления $^{131}J—T_3$ в ходе дейодирования $^{131}J—T_3$, связывающая способность отдельных фракций смещается от TB*G* к TBPA. Ввиду того, что — в противоположность принципиальным основам метода разведения изотопа

— меченое вещество и нерадиоактивный T_4 отчасти неодинаковы, кажется вероятным, что измеренная радиоактивность не представляет фактических условий связывания T_4.

Авторы проводили также исследование транспорта, определили величины теста Гамолского, в той же сыворотке крови. Результаты показали лишь незначительные колебания и $^{131}J—T_3$ также оказался значительно более устойчивым.

По мнению авторов тест Гамолского довольно надежно применим для характеристики функции щитовидной железы, тогда как изучение способности фракций белков крови к связыванию T_4 методом разведения изотопа дает ненадежные результаты и его клиническая и терапевтическая ценность сомнительны. Правильно было бы поставлять $^{131}J—T_4$ в пределах нескольких дней после производства в субстанции и использовать препарат при разведении ex tempore, быть может, применять его при двойном мечении.

ИЗУЧЕНИЕ МЕХАНИЗМА ДЕЙСТВИЯ ДИЙОДТИРОЗИНА И ЙОДА

Я. ФЁЛДЕШ, Е. ГЕСТЕШИ и Й. ЮХАС

В целях уяснения механизма действия дийодтирозина авторами были проведены модельные опыты на животных и сравнения результатов с данными, полученными при помощи KJ. Согласно результатам, ни один из изученных препаратов не задерживает стимулирующее действие гормона, освобождающего тиреотропный гормон, на экскреуню ТСГ. В остром опыте KJ и дийодтирозин не изменяют чувствительности щитовидной железы к ТСГ и к LATS. На основе исследований авторов большая доза дийодтирозина несколько замедляет исчезновение меченного тироксина из кровообращения, в то время как KJ не оказывает такого действия. Подробно обсуждается значение этого наблюдения.

FIFTH INTERNATIONAL CONGRESS OF THE EUROPEAN SOCIETY OF PATHOLOGY

Vienna, October 6—10, 1975

Preliminary program
Monday, Oct. 6

10.00—11.00 Opening ceremony
11.30—12.30 Guest lecture (K. Lorenz)
14.30—17.30 Parallel sessions:
- A: Gastrointestinal Pathology, including endoscopic technics
- B: Perinatal Pathology, including placental pathology
- C: Free paper session
- D: Slide Seminar: ear, nose and throat

Tuesday, Oct. 7

09.00—10.30 Modern aspects of inflammation
11.00—12.30 Virus and immunopathology
14.30—17.30 Parallel sessions:
- A: Immunopathology (Immunodeficiencies, Methodology)
- B: Functional evaluation of testicular biopsies
- C: Free paper session
- D: Slide seminar: gastrointestinal biopsies

09.00—10.20 Pathology of Transplantation
11.00—12.30

Thursday, Oct. 9

09.00—10.30 Pathology due to pollution
11.00—12.00 Modern ideas of the structure of chromosomes (Symeonide lecture)

14.30—16.30 Parallel sessions:

A: Prenatal diagnosis

B: Inflammation complements (including infections by unusual microorganisms)

C: Free paper session

D: Slide seminar: Dermatohistopathology

17.00—18.00 General Assembly of the European Society of Pathology

Friday, Oct. 10

09.00—10.30 Pathology and classification of lymphomas

14.30—17.30 Parallel sessions:

A: Pathology of chromosomes

B: Retrieval in Pathology

C: Free paper session

D: Slide Seminar: diseases of the lymphatic tissue

VTH EUROPEAN CONGRESS OF THE EUROPEAN SOCIETY OF PATHOLOGY

Office of Secretariat: Istituto di Anatomia Patologica Via F. Sforza 38 20122 Milano

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I am writing on behalf of the Organizing Committee of the Vth European Congress of Pathology to inform you that the European Society of Pathology will be holding its Fifth Congress in Vienna from October 6—10, 1975.

The Congress Committee is anxious to ensure that as many European pathologists as possible receive early notice of tha event so that they are able to schedule their attendance at the Congress into their 1975 programme. The Committee would therefore be grateful if you would refer to the Congress in any publications which you sponsor, particularly in your list of coming meetings. The Preliminary Programme may assist you in this regard.

All interested persons who write to be placed on our mailing lists, will be sent a Registration Form and Advance Programme towards the end of 1974.

May I request that you acknowledge this letter by completing and returning the tear-off slip below.

Thank you, yours sincerely,

A. GIORDANO

Secretary General

Secretary Office: European Society of Pathology — Via F. Sforza 38 — Milano

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11. III. 1975 Index: 26.016

ACTA MEDICA

ACADEMIAE SCIENTIARUM HUNGARICAE

TOMUS XXXI FASCICULI 3—4

AKADÉMIAI KIADÓ, BUDAPEST

1974

ACTA MEDICA

A MAGYAR TUDOMÁNYOS AKADÉMIA ORVOSTUDOMÁNYI KÖZLEMÉNYEI

KIADÓHIVATAL: BUDAPEST V., ALKOTMÁNY UTCA 21.

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (3—4), pp 139—145 (1974)

HAEMODYNAMIC RESPONSES TO DROTAVERINE AND NORADRENALINE IN THE DOG

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Received January 10, 1974

Lymph stasis in the tissues and oedematous imbibition of the vascular walls was induced by the joint administration of noradrenaline and drotaverine. In consequence, volume hypertension, increase in central venous pressure and an enhancement of lymph flow developed.

Massive doses of noradrenaline are known to produce generalized fibrinoid necrosis of the vascular walls [5, 9, 12], an observation confirmed by our earlier studies Jellinek et al. It has been shown by other workers that the necrosis of tissues including the vessel walls induced by catecholamines, in particular by noradrenaline, are brought about by an adrenergic alpha and beta minuting effect [6, 7]. In view of the fact that beta receptor stimuation results in spastic smooth-muscle contraction, in an earlier study it was attempted to prevent the noradrenaline induced necrosis by means of the spasmolytic agent drotaverine. This drug has indeed proved to prevent the necrosis. On the other hand, the combined administration of noradrenaline and drotaverine was followed by lymph stasis and oedematous imbibition of the vessel walls [3]. Though our earlier studies [3, 4] had been focussed on the catecholamine-induced changes of myocardium and coronaries, the liver, kidney, pancreas, skeletal muscles and intestine also revealed similar changes. Distension of a large number of lymph capillaries in the test group was a clear sign of lymph stasis (Figs 1, 2). The present study has been concerned with these changes and the haemodynamic alterations involved in their production.

Material and method

Fifty-three mongrel dogs of either sex, weighing between 7 and 17 kg, were used. Under 100 mg/kg chloralose anaesthesia catheters were introduced into the femoral vein and artery. Central venous and arterial pressures were recorded continuously, cardiac output was estimated on the basis of Fick's principle in 15 minute-intervals. Lymph flow was measured through a cannula tied into the thoracic duct by suction at 10 cm H_2O.

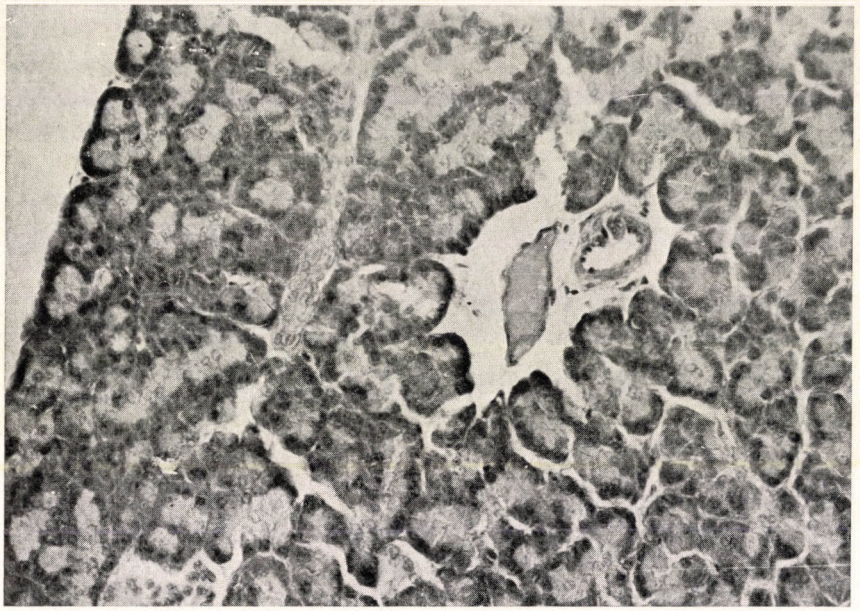

Fig. 1. Distended lymph vessel in the pancreas. PAS staining

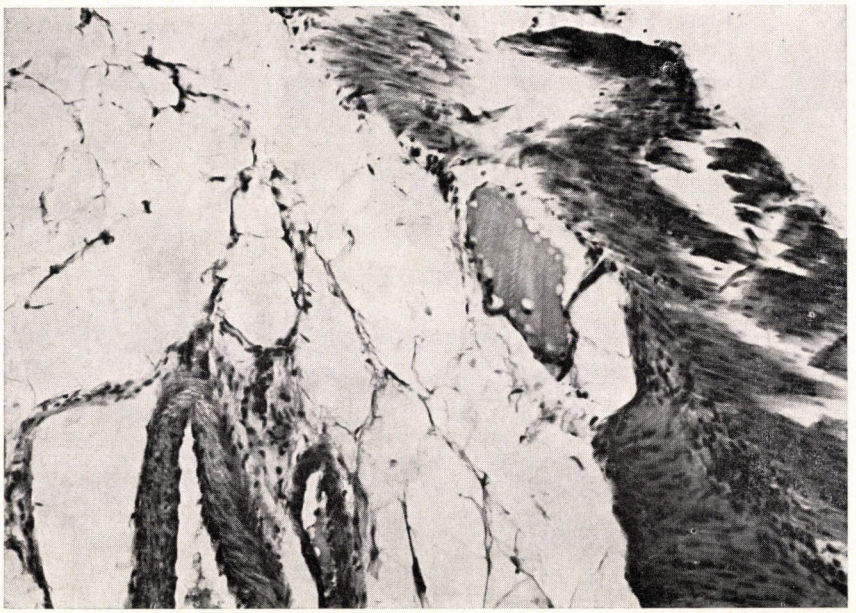

Fig. 2. Distended lymph vessel in the mesentery. HE staining

Noradrenaline was administered in doses of 10 μg/kg/min, drotaverine in doses of 200 μg/kg/min by intravenous infusion in 0.4 ml/kg/min physiological saline, for 45 min. The controls received physiological saline only. Statistical evaluation was done by analysis of variance, deviations are given in values of the standard error.

Results

Fig. 3 shows the changes in arterial mean pressure. It can be seen that the hypertensive effect of noradrenaline was scarcely affected by drotaverine given simultaneously.

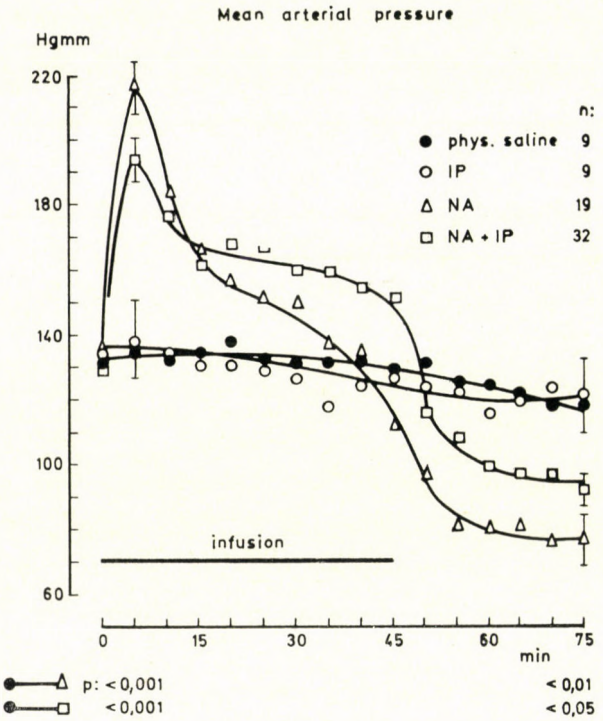

Fig. 3. Changes in the arterial mean pressure. In this and in the following figures the mark "n" shows the number of experiments, "p" the degree of significance, "IP" the results of the animals treated with drotaverine, "NA" the results of the animals treated with noradrenaline, "NA-IP" the results of the animals treated with the combination of drotaverine and noradrenaline and "phys. saline" the result of the control animals

Figs 4 and 5 show the cardiac output and the calculated peripheral resistance. Noradrenaline by itself increased the peripheral resistance and reduced cardiac output significantly. The combined administration of noradrenaline and drotaverine caused a marked elevation of cardiac output without affecting peripheral resistance. In other words, the noradrenaline induced resistance-hypertension was converted to a volume-hypertension. At the same

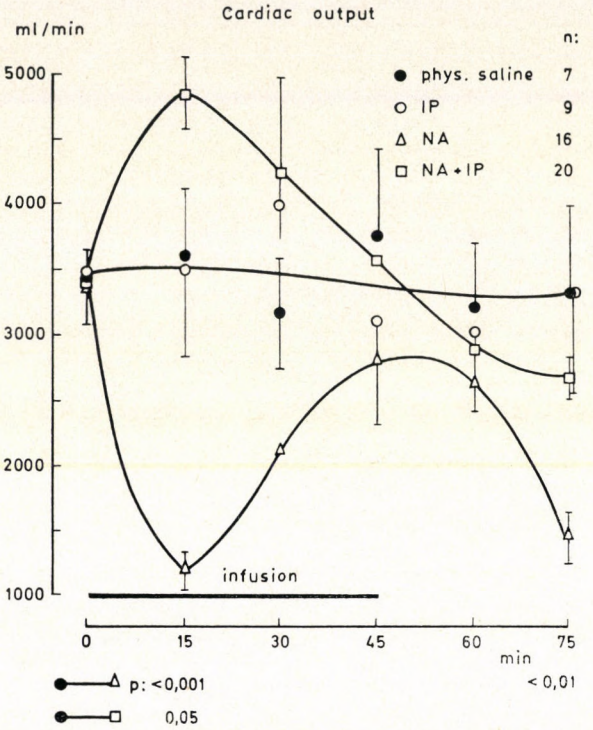

Fig. 4. Changes in the cardiac output. Marks as in the figure 3

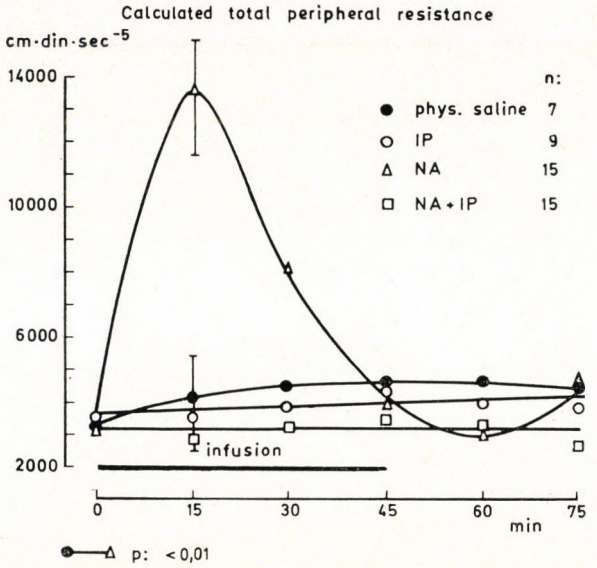

Fig. 5. Changes in the calculated total peripheral resistance. Marks as in the figure 3

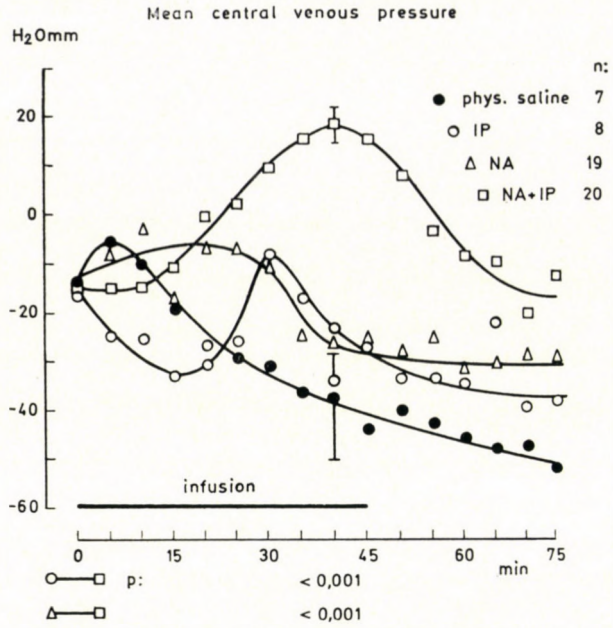

Fig. 6. Changes in the mean central venous pressure. Marks as in the figure 3

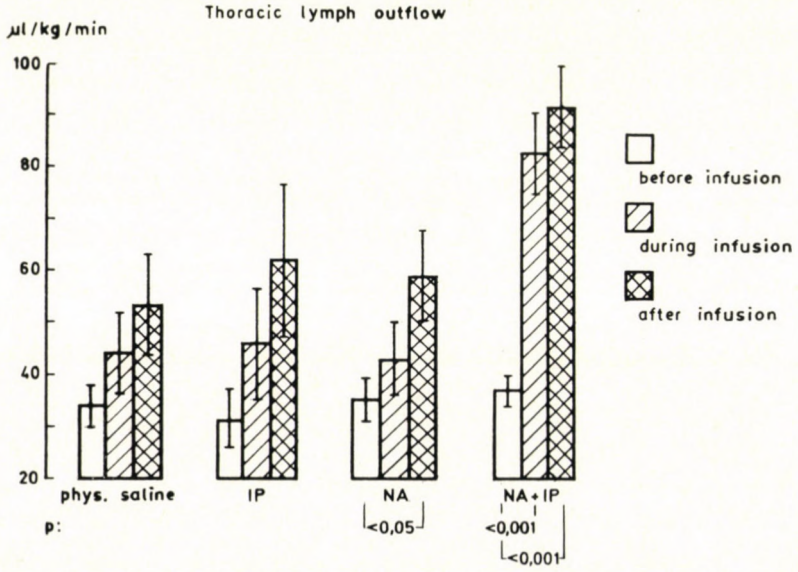

Fig. 7. Changes in the lymph outflow from the thoracic duet

time the changes in cardiac output were due to changes in stroke volume, as no significant change in heart frequency occurred.

Fig. 6 shows mean central venous pressure. Treatment with noradrenaline and drotaverine was followed by a significant increase in central venous pressure. When administred by themselves the drugs, venous pressure was unaffected.

In Fig. 7 the changes in thoracic duct lymph outflow are seen. Combined administration of the two drugs elicited a significant increase in lymph outflow. When the drugs were administred by themselves, lymph flow, though rising to some extent, showed no significant difference from the control values.

Discussion

In agreement with the present findings, lymph stasis in the tissues in response to the joint administration of adrenaline and caffeine was noted by AMOSSOW [1] and RUKONEV et al. [11], an observation attributed by the authors to an enhanced outflow of plasma. As regards the mechanism of the process, the views are divided while MILLER et al. [8] and POSCHE [10] regard hypoxia as the primary factor of enhanced plasma outflow responsible for lymph stasis, other authors have failed to note any consistent increase in plasma outflow in hypoxia which might have led to lymph retention [2]. On the evidence of our observations catecholamines, unless administered together with drotaverine, fail to enhance plasma outflow to the extent of involving an oedema of the vessel walls. On the other hand, catecholamines alone are also capable of eliciting hypoxia in the tissues. Consequently, the hypoxia cannot be responsible for the production of lymph retention. It seems likely that haemodynamic changes, in particular an increased blood flow in the tissues and a transitory rise in central venous pressure, play a definite part in the increase in plasma outflow and the resulting lymph stasis. Clarification of this mechanism awaits further studies.

It also emerged that potent spasmolytic agent, while being capable of relieving the noradrenaline induced vasoconstriction, fails to counteract the effect of noradrenaline enhancing heart contractions. This was indicated by the rise in stroke volume, as a sign that the resistance hypertension had been converted to a volume hypertension. Whether this observation is likely to bear fruit in human pathology, for instance in the management of cardiogenic shock, is a question to be elucidated by future studies.

REFERENCES

1. AMOSSOW, M. G.: **3,** 321 (1969).
2. BÜCHNER, F., ONISHI, S.: Verh. dtsch. Ges. Path. **51,** 139 (1967) and Beitr. path. Anat. **135,** 153 (1967).
3. HÜTTNER, I., KERÉNYI, T., VERESS, B., JELLINEK, H., POGÁTSA, G., GÁBOR, G.: Frankf. Z. Path., **76,** 107 (1967).
4. JELLINEK, H., HÜTTNER, I., KERÉNYI, T., GÁBOR, G., POGÁTSA, G.: Acta morph. Acad. Sci. hung. **14,** 183 (1966).
5. KLINE, J. K.: Amer. J. Path. **38,** 539 (1960).
6. MALING, H. M., HIGHMAN, B.: Amer. J. Physiol. **194,** 590 (1958).
7. MÉHES, G., PAPP, G., RAJKOVITS, K.: Acta physiol. Acad. Sci. hung. **32,** 175 (1967).
8. MILLER, A. J., PICK, R. and KATH, L. N.: Circulat. Res. **8,** 941 (1960) and Circulation **29,** 485 (1964).
9 PEARCE, R. M.: J. exp. Med., **8,** 400 (1906).
10. POSCHE, R.: Verh. dtsch. Ges. Path. **49,** 219 (1965).
11. RUKONEV, U. S.: Arch. Path. **26/5,** 381 (1964) and Kardiologija **8/9,** 54 (1968).
12. SZAKÁCS, I. E., CANNON, A.: Amer. J. clin. Path. **30,** 425 (1958).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (3—4), pp 147—155 (1974)

ÜBER DIE WIRKUNG VON FRUKTOSE AUF DEN HARNSÄUREMETABOLISMUS

Von

Erzsébet HOLLÄNDER

II. INTERNISTISCHER LEHRSTUHL INSTITUT FÜR ÄRZTLICHE FORTBILDUNG, BUDAPEST

(Eingegangen am 14. Januar 1974)

Unter Wirkung der Fruktoseinfusion steigt bei Normalpersonen und bei Gichtkranken der Harnsäuregehalt im Plasma und im Harn an. Der Harnsäureüberschuß wird bei Gichtkranken langsamer ausgeschieden als bei Normalpersonen und der Harnsäurespiegel im Plasma ist sogar vier Stunden nach der Fruktosezufuhr erhöht.

Bei mit Oxonsäure urikasegehemmten Ratten können ähnliche Veränderungen des Plasma-Harnsäurespiegels beobachtet werden wie bei Gichtkranken. Die sich unter Fruktosewirkung entwickelnde Hyperurikämie findet ihre Erklärung in den intermediären Stoffwechselveränderungen und der in der Begleitung von Dehydration verlaufenden, nicht fruktosebedingten, gesteigerten Diurese.

Nebst der angeborenen Störung des Harnsäurestoffwechsels unbekannter Ätiologie, gibt es bekanntlich zahlreiche pathologische Zustände, in denen der Harnsäuregehalt des Plasmas auf Wirkung einiger Nährstoffe und Medikamente ansteigt. TALBOTT [22] beschrieb die hyperurikämisierende Wirkung der purinreichen Speisen und alkoholischen Getränke. Nach der Beobachtung von MCLACHLAN und RODNAN [14] kann nicht nur eine reichliche Ernährung, sondern auch das Hungern Gicht provozieren. Über den, von der Dosis abhängigen, Plasma-Harnsäurespiegel-steigernden Effekt der Salizylate haben YÜ und GUTMAN [25] und über die hyperurikämisierende Wirkung der Thiazidderivate DEMARTINI und WHEATON [9] sowie WYNGAARDEN [24] berichtet. Die Gichtanfallauslösende Wirkung anderer Diuretika ist auch bekannt [10]. COHEN [7] berichtete über einen Fall, in dem durch Zoxazolamin eine harnsäurebedingte Nierenschadigung verursacht wurde. Der den Harnsäurespiegel steigernde Effekt der Nahrung beruht auf Purinzufuhr, während durch die erwähnten Medikamente die renale Harnsäureausscheidung gestört wird. Die sich auf diese Weise entwickelte vorübergehende Hyperpurikämie kann sowohl bei normalem, als auch bei pathologischem Harnsäurestoffwechsel beobachtet werden. Zur Differenzierung des physiologischen und des pathologischen Harnsäurestoffwechsels eignen sich aber weder die durch die mit der Nahrung zugeführten Harnsäure-Präkursoren, noch die durch Hemmung der Harnsäureausscheidung herbeigeführte Hyperurikämie. Im Laufe unserer früheren Untersuchungen trachteten wir den Unterschied zwischen normalen und pathologischen Zuständen anhand der nach intravenöser Verabreichung von Furosemid entstandenen Änderungen der Plasma-

und Harn-Harnsäurekonzentration zu klären, unsere Ergebnisse waren aber nicht überzeugend.

Angesichts der bekannten Tatsache, daß in der Gichtpathogenese nebst der gestörten Harnsäureausscheidung auch die gesteigerte Produktion eine wichtige Rolle spielt, wählten wir zu unseren Experimenten eine natürliche Substanz aus, die am intermediären Stoffwechsel der Harnsäure beteiligt ist. Über die zu diesem Zweck geeignet scheinende Fruktose ist es bekannt, daß sie eine Herabsetzung des organischen Phosphatgehalts und eine Steigerung des Glukose- und Milchsäuregehalts im Plasma herbeiführt [3, 4, 13, 16, 17].

Material and Methodik

Die Untersuchungen fanden bei 6 unbehandelten Gichtkranken und 6 normourikämischen gesunden Personen statt. Da es sich im Laufe der Vorversuche herausstellte, daß durch 1 g/kg der 20%-igen Fruktoselösung keinerlei Schädigung verursacht wird, haben wir die Dosis — um eine ausdrücklichere Stoffwechselantwort zu erhalten — auf 2 g/kg erhöht. Die Fruktoseverabreichung erfolgte in Infusion, deren Dauer sich auf 30 Minuten belief. Vor der Infusion durften die Probanden keine Nahrung zu sich nehmen, das Wassertrinken war aber unbeschränkt. Im Laufe der der Infusion vorangegangenen 3 Tage erhielten die untersuchten Personen eine etwa 2000 Kalorien enthaltende purinarme Diät, den Gichtkranken wurden in dieser Zeit keine den Harnsäurestoffwechsel modifizierende Medikamente verabreicht. Blutentnahme und Harnsäurebestimmungen fanden vor der Infusion sowie am Ende der 1., 2. und 4. Stunde statt.

Die basale Harnsäureausscheidung der Patienten und der Kontrollpersonen wurde im vor der Infusion 4 Stunden lang gesammelten Harn bestimmt. Von Infusionsbeginn an wurde der Harn wieder 4 Stunden lang gesammelt. Die Plasma- bzw. Harn-Harnsäure-Bestimmung erfolgte mit der Methode von CARAWAY [6].

Zur Ergänzung dieser Untersuchungen wurde die auf den Harnsäurestoffwechsel ausgeübte Wirkung von Fruktose auch bei 4 intakten und 4 urikasegehemmten, 250—300 g wiegenden R-Amsterdam-Rattenmännchen untersucht. Die die Urikase (Serva Feinbiochemica) reversibel lähmende Oxonsäure wurde auf den Vorschlag von BODA und Mitarb. [1] nach dem Verfahren von BRANDENBERGER [5] hergestellt. Die die Hemmung der harnsäurespaltenden Wirkung der Urikase haben wir mit Oxonsäure auch in vitro kontrolliert.

An den dem Experiment vorangehenden 4 Tagen erhielten die Tiere 3×20 mg/Tag Oxonsäure intraperitoneal. Nachdem aus der Schwanzvene Blut entnommen wurde, kam es zur intraperitonealen Verabreichung von 3 g/kg Fruktose. Nach 1, 2, 4 und 12 Stunden erfolgten wieder Blutentnahme und Harnsäurebestimmung.

Zur Eliminierung der die molare Diurese beeinflussenden Rolle von Fruktose wurden bei einer anderen Gruppe der Tiere die A. und V. renalis unterbunden. Vor der Unterbindung der Nierengefäße erhielten 4 Tiere vier Tage lang die oben beschriebene Oxonsäurebehandlung, während bei 4 weiteren Tieren die Urikase nicht gehemmt wurde. 2 Stunden nach dem Eingriff wurde sämtlichen Tieren intraperitoneal 3 g/kg Fruktose verabreicht, während 4 Kontrolltiere nach Unterbindung der A. und V. renalis in identischer Menge 0,9%-ige Kochsalzlösung erhielten. Blutentnahme und Harnsäurebestimmung fanden in der oben beschriebenen Weise statt.

Ergebnisse

Beim Menschen hat sich nach der Infusion von 2 g/kg Fruktose die Harnsäurekonzentration des Plasmas in sämtlichen Fällen erhöht. Während sich die maximale Wirkung in beiden Gruppen am Ende der 1. Stunde meldete,

waren — was Ausmaß der Erhöhung und Regression der Kurve anbelangt — zwischen den Gichtkranken und den Kontrollpersonen Abweichungen festzustellen.

Der bei den 6 unbehandelten Gichtkranken registrierte hohe Nüchtern-Plasmaharnsäure-Spiegel (9,3 $\pm$ 0,55 mg%) hat sich binnen 1 Stunde auf 11,3 $\pm$ 1,0 mg% erhöht und bis zum Ende der 2. Stunde nur um 0,1 mg%

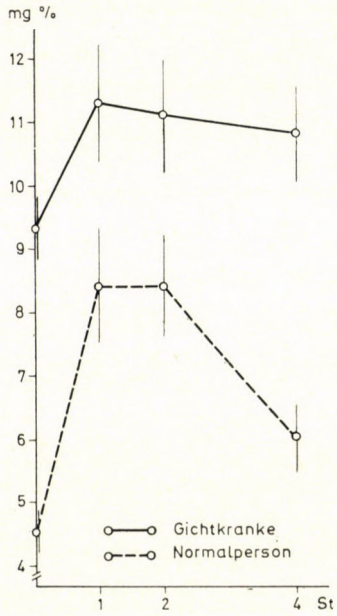

Abb. 1. Plasma-Harnsäurekonzentration bei Gichtkranken und Normalpersonen

vermindert; Ende der 4. Stunde lag die Harnsäurekonzentration noch immer bei 10,8 $\pm$ 0,8 mg%. Die Harnsäurekurve der Kontrollpersonen ging von 4,5 $\pm$ 0,3 mg% aus und stieg rasch an; bis zum Ende der 1. Stunde erhöhte sich der Wert auf 8,4 $\pm$ 0,9 mg% und blieb auch in der 2. Stunde auf diesem Niveau. Ende der 4. Stunde widerspiegelte die steil sinkende Kurve die rasche Verminderung der Werte, bis die Plasma-Harnsäurekonzentration abermals in der Nähe des Normalbereichs lag: 5,95 $\pm$ 0,6 mg% (Abb. 1).

Durch die Infusion der 20%-igen Fruktoselösung wurde in sämtlichen Fällen eine reichliche molare Diurese ausgelöst, zu der sich die Verringerung der Harnsäurekonzentration gesellte. Die in der Untersuchungsperiode ausgeschiedene Harnsäuremenge erhöhte sich bei den Kontrollpersonen auf das 3,4 fache und bei den Gichtkranken auf das Doppelte der Normalausscheidung (Tab. I).

Die Gestaltung der Plasma- und Harn-Harnsäurekonzentration nach der Fruktoseinfusion haben wir auch bei 6 mit Allopurinol behandelten Gichtkranken untersucht. Nennenswerte Änderungen waren weder im Plasma, noch im Harn vorzufinden: Die Plasma-Harnsäurekonzentration erhöhte sich um 0,1—0,2 mg% und der Harn-Harnsäuregehalt um 40—50 mg.

Durch Urikasehemmung konnten bei Ratten dem menschlichen Harnsäurestoffwechsel ähnliche Verhältnisse herbeigeführt werden. Am Ende der 1.

Tabelle I

Wirkung der Fruktoseinfusion auf den Harnsäure-Gehalt des Harns

	Harnmenge ml	Konzentration mg%	Ausgeschiedene Harnsäuremenge mg
Kontrollgruppe			
Vor der Fruktose-infusion	320	24	76,8
Nach der Fruktose-infusion	1500	16	260
Gichtgruppe			
Vor der Fruktose-infusion	250	37	92,5
Nach der Fruktose-infusion	940	19	178,6

auf die Fruktoseinjizierung folgenden Stunde war in sämtlichen Fällen eine plötzliche Erhöhung des Plasma-Harnsäurespiegels zu registrieren. In der 2. Stunde meldete sich eine mäßige Verminderung, der 4stündige Wert erreichte annähernd wieder das 1stündige Maximum, um sich danach stufenweise zu verringern; auf den Ausgangswert kehrte die Harnsäurekonzentration erst in der 12. Stunde zurück (Abb. 2).

Bei den mit Oxonsäure nicht behandelten Tieren ließ sich keine Erhöhung des Blut-Harnsäuregehalts beobachten. Die Kurve der unter Fruktosewirkung eingetroffenen Änderungen der Plasma-Harnsäurewerte ist den bei den Gichtkranken registrierten Abweichungen ähnlich, ein Unterschied läßt sich nur beim anfänglichen plötzlichen Anstieg erkennen. Als wahrscheinliche Ursachen der Abweichung kommen artbedingte Eigenschaften, größere Fruktosedosen sowie die kontinuierliche, den Harnsäuremetabolismus modifizierende Oxonsäurebehandlung in Frage.

Nach Unterbindung der A. und V. renalis stieg der Plasma-Harnsäuregehalt unter Fruktosewirkung plötzlich an und erreichte den Höchstwert in

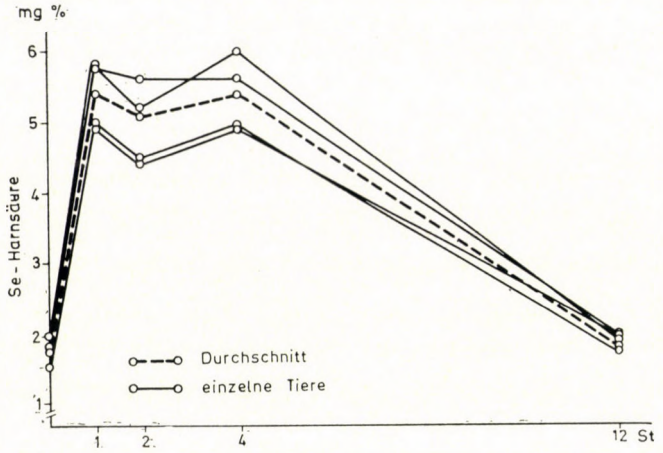

Abb. 2. Plasma-Harnsäurekonzentration bei vier Tage lang mit Oxonsäure [3 × 20 mg/Tag] vorbehandelten Ratten nach intraperitonealer Injizierung von 3 g/kg Fruktose

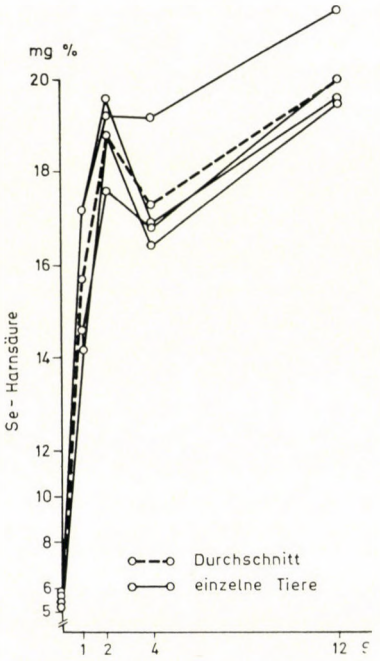

Abb. 3. Wirkung von 3 g/kg Fruktose auf den Plasma-Harnsäuregehalt bei urikasegehemmten Ratten nach Unterbindung der A. und V. renalis

der 2. Stunde. Ende der 4. Stunde meldete sich eine vorübergehende Verminderung, worauf sich parallel mit der Entwicklung des urämischen Zustandes wieder eine langsame Erhöhung in Gang setzte. Bei der Dekapitation der

Tiere in der 12. Stunde betrug der Plasma-Harnsäuregehlt im Durchschnitt 20 mg% (Abb. 3). Bei der Sektion zeigte sich keine Nahtinsuffizienz, die Nieren waren vergrößert, das Parenchym blutig imbibiert und die Struktur verschwommen.

Nach Unterbindung der Nierengefäße erhöhte sich der Plasma-Harnsäurespiegel auch bei den Kontrolltieren (Abb. 4), wofür der akute urämische Zustand verantwortlich war. Die am Ende der 1. Stunde beobachteten Maximal-

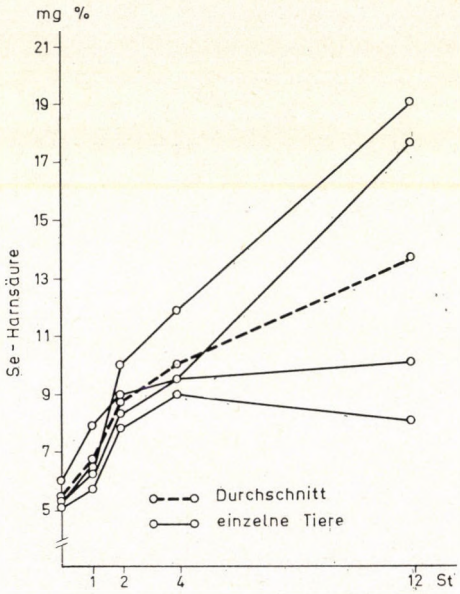

Abb. 4. Plasma-Harnsäurekonzentration bei urikasegehemmten Kontrolltieren nach Unterbindung der A. und V. renalis

werte, die sowohl bei den Tieren mit unterbundenen Nierengefäßen, als auch bei den intakten und mit Fruktose behandelten Ratten zu registrieren waren, entwickelten sich aber bei den Kontrolltieren nicht.

Besprechung

In bezug auf die Auswirkungen des Fruktosemetabolismus finden sich in der Literatur widerspruchsvolle Angaben. PERHEENTUPA und RAIVIO [18] beobachteten nach der i. v. Zufuhr von 0,5 g/kg Fruktose nicht nur bei hereditärer Fruktose-Intoleranz, sondern auch beim Normalmenschen die Erhöhung des Plasma- und Harn-Harnsäuregehalts. CURRERI und PRUITT [8] fanden nach der intravenösen Verabreichung von 100 g Fruktose keine diesbezügliche

Änderung, ebenso wie SAHEBJAMI und SCALETTER [20] die nach der Fruktoseinfusion sowohl bei Normalpersonen als auch bei hereditärer Fruktose-Intoleranz unveränderte Plasma-Harnsäurewerte registrierten. Laut HEUCKENKAMP und ZÖLLNER [11] steigt bei Normalpersonen nach Verabreichung von 1,5 g/kg Fruktose die Harnsäurekonzentration im Plasma an.

Im Falle eines normalen Stoffwechsels wird Fruktose im Organismus teils zu Glukose, teils zu Milchsäure metabolisiert. Bei den Säugern spielt sich die Fruktoseumwandlung im Falle einer intakten Leberfunktion rasch ab; die erste Stufe des Abbaus ist ein von der Insulinwirkung unabhängiger Prozeß [19, 23]. Fruktose wird in der Leber durch Fruktose-1-Phosphat abgebaut und zwar teils zu Glyzeraldehyd, teils zu Dihydroxyacetonphosphat. Die sich im Laufe des weiteren Abbaus von Glyzeraldehyd bildenden Produkte werden durch den Organismus in der Glukoneogenese verwendet. Die mit der Bildung von Dihydroxyacetonphosphat beginnende Reaktion verläuft in Richtung der anaeroben Glykolyse. Das Endprodukt des Prozesses ist Milchsäure. Durch die rasche Zufuhr großer Fruktosedosen wird die Vermehrung der Endprodukte beider Abbauprozesse herbeigeführt. Die durch die gesteigerte Milchsäurebildung ausgelöste Azidose könnte für die auf Wirkung der Fruktoseinfusion entstandene Hyperurikämie eine Erklärung liefern.

Es ist nicht wahrscheinlich, daß Fruktose oder Milchsäure die Purinsynthese direkt fördern würden. Die Hyperurikämie entwickelt sich nur 1 Stunde nach der Fruktosezufuhr, durch die wirksamen Stimulatoren der Purinsynthese wird der Harnsäuregehalt des Plasmas dagegen nur nach 24—48 Stunden gesteigert [12, 21].

Eine andere Erklärung der beim Menschen und bei der Ratte durch Fruktoseinfusion herbeigeführten Hyperurikämie könnte sein, daß durch Fruktose der Zellstoffwechsel geändert wird. Die Phosphorylierung von Fruktose verläuft unter Verwednung von ATP. Durch die Verringerung des ATP-Gehalts werden indessen die, die Purin-Nukleotiden beschleunigenden Prozesse aktiviert und des gesteigerten Purinabbaus zufolge steigt der Harnsäuregehalt des Organismus an. Diese Theorie wird auch durch die Beobachtungen von MAENPAA und RAIVIO. [15] sowie BODE und Mitarb. [2] unterstützt, laut der sich der unorganische Phosphorgehalt des Bluts während der Fruktoseinfusion verringert.

Unsere Experimente haben bewiesen, daß die diuretische Wirkung der hypertonischen Fruktose in der Entstehung der Hyperurikämie keine Rolle spielt. Für die anfängliche hohe Harnsäurekonzentration können osmotische Änderungen, wie z. B. eine hypertonische Dehydration verantwortlich sein, für die weitere Gestaltung liefern aber diese Prozesse allein keine genügende Erklärung. Über eine bedeutende und andauernde extrazelluläre Dehydration kann angesichts der Änderung des Hämatokritwertes nicht die Rede sein — da sich in der 4. Stunde der Ausgangswert von 45,2 $\pm$ 0,78% auf 49,2 $\pm$ 2,33%

erhöhte (der Unterschied ist nicht signifikant, $p < 0{,}2$). Nach dem Unterbinden der A. und V. renalis steigt parallel mit der Entwicklung der akuten Urämie der Harnsäuregehalt im Plasma an; wenn die Tiere auch Fruktose erhalten, ist der anfängliche Anstieg ausgeprägter, welcher Umstand nicht einfach als die Folgeerscheinung der Urämie oder des Operationsschocks bzw. des Gewebetraumas betrachtet werden kann.

Unter unseren anläßlich der Untersuchung der Wechselwirkungen der Fruktose- und Harnsäurestoffwechsel ermittelten Beobachtungen halten wir folgende für wichtig:

1. Unter Wirkung der Fruktoseinfusion steigt sowohl bei unbehandelten Gichtkranken als auch bei Normalpersonen der Harnsäuregehalt des Plasmas an. Bei den Gichtkranken war der Harnsäurespiegel auch in der vierten Stunde nach der Fruktosezufuhr erhöht.

2. Die Niere des gesunden Menschen scheidet den sich auf diese Weise entwickelten Harnsäureüberschuß rasch aus, die Niere des unbehandelten Gichtkranken ist aber zur raschen Ausscheidung des Harnsäure-Überschusses unfähig.

3. Im Laufe der die Harnsäuresynthese hemmenden Behandlung (Allopurinol) blieb der Harnsäuregehalt von Plasma und Harn unverändert.

4. Bei mit Oxonsäure urikasegehemmten Ratten können nach Fruktosegabe ähnliche Änderungen der Plasma-Harnsäure beobachtet werden, wie bei den Gichtkranken.

5. Die die Plasma-Harnsäurekonzentration steigernde Wirkung von Fruktose kann mit der konsekutiv erhöhten Diurese bzw. der Hypovolämie und Dehydration nicht erklärt werden. Nach dem Unterbinden der A. und V. renalis entwickeln sich bei den urikasegehemmten Ratten unter Fruktosewirkung ähnliche Veränderungen wie bei den nicht operierten Tieren. Für den hochgradigen Anstieg des Plasma-Harnsäuregehalts sind in diesen Fällen zum Teil auch die Urämie, der Operationsschock und die Gewebeschädigung verantwortlich.

LITERATUR

1. BODA, D., PÉNZES, P. und Mitarb.: Modellkísérletek az emberi shockvese pathomechanizmusának kiderítésére. Orv. Hetil. **111,** 2354 (1970).
2. BODE, L., SCHUMACHER, H. und Mitarb.: Fructose-induced depletion of liver adenine nucleotides in man. Hormone Metab. Res. **3,** 71 (1971).
3. BERGSTRÖM, J., HULTMAN, E.: Synthesis of muscle glycogen in man after glucose and fructose infusion. Acta med. scand. **182,** 93 (1967).
4. BEGSTRÖM, J., ROCH-NORLUND, A. E.: Lactic acid accumulation in connection with fructose infusion. Acta med. scand. **184,** 359 (1968).
5. BRANDENBERGER, H.: The oxidation of uric acid to oxonic acid and its application in tracer studies of uric biosynthesis. Biochim. biophys. Acta (Amst.) **15,** 108 (1954).
6. CARAWAY, W. T.: Uric acid. Standard methods in clinical chemistry. New York, Academic Press **4,** 239 (1963) .
7. COHEN, T.: Nephropathy associated with the oral administration of zoxazolamine. New Engl. J. Med. **256,** 1193 (1957).

8. CURRERI, P. W., PRUITT, B. A.: Absence of fructose-induced hyperuricaemia in man. Lancet. **1,** 839 (1970).
9. DEMARTINI, F. E., WHEATON, E. A.: Effect of chlorothiazide on the renal exrection of uric acid. Amer. J. Chem. **32,** 572 (1962).
10. GOODMAN, L. S., GILMAN, A.: The pharmacological basis of therapeutics. 3. ed. Macmillen Co. Toronto 1965.
11. HEUCKENKAMP, P. U., ZÖLLNER, N.: Fructose-induced hyperuricaemia Lancet. **1,** 808. (1971).
12. KRAKOFF, I. H., BALIS, M. E.: Studies on 2-substituted thiadiazoles in man. J. clin. Invest. **38,** 907 (1957).
13. LEVIN, B., SNODGRASS, J. A. I.: Fructosaemia. Observations of seven cases. Amer. J. Med. **45,** 826 (1968).
14. MACLACHLAN, M. J., RODNAN, G. P.: Effects of food, fast and alcohol on serum uric acid and acute attacks of gout. Amer. J. Med. **42,** 38 (1967).
15. MAENPAA, P. H., RAIVIO, K. O.: Liver adenine nucleotides: Fructose-induced depletion and its effect on protein synthesis. Science **161,** 1253 (1968).
16. MILLER, M., CRAIG, J. W. und Mitarb.: The metabolism of fructose in man. Yale J. biol. Med. **29,** 335 (1956).
17. MORRIS, R. C.: An experimental renal acidification defect in patients with hereditary fructose intolerance. Its resemblance to renal tubular acidosis. J. clin. Invest. **47,** 1389 (1968).
18. PERHEENTUPA, J., RAIVIO, K.: Fructose-induced hyperuricaemia. Lancet **2,** 528 (1967).
19. RAIVIO, K., KEKOMAKI, M. P.: Depletion of liver adenine nucleotides induced by D-fructose. Biochem. Pharmacol. **18,** 2615 (1968).
20. SAHEBJAMI, H., SCALETTER, R.: Effects of fructose infusion on lactate and uric acid metabolism. Lancet **1,** 366 (1971).
21. SEEGMILLER, J. E., GRAYZELL, A. I. und Mitarb.: The effect of 2-ethylamino-1,3,4-thiadiazole on the incorporation of glycine into urinary purines and uric acid in man. Metabolism **12,** 507 (1963).
22. TALBOTT, J. H.: Gout. 3rd ed. Grune and Stratton, New York 1964.
23. WOODS, H. F., EGGLESTON, L. V.: The cause of hepatic accumulation of fructose-1-phosphate on fructose loading. Biochem. J. **119,** 501 (1970).
24. WYNGAARDEN, J. B.: The pathophysiology of hyperuricaemia and gout. Univ. Michigen med. Centr. **10,** 260 (1968).
25. YÜ, T. F., GUTMAN, A. B.: Study of the paradoxical effects of salicylate in low, intermediate and high dosage on the renal mechanism for excretion of urate in man. J. clin. Invest. **38,** 1298 (1959).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (3—4), pp 157—163 (1974)

EFFECT OF PROSTAGLANDINS AND DROTAVERINE ON ADP-INDUCED PLATELET AGGREGATION

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Received March 14, 1974

Prostaglandings E_1 and E_2 have been studied for their effect on platelet aggregation *in vitro*. PGE_1 proved a highly potent, PGE_2 a slightly less potent inhibitor of platelet aggregation. The two prostaglandins were also studied in combination with drotaverine the inhibitory activity of which against ADP-induced platelet aggregation had been found to function at high concentrations only. Both combinations revealed a potentiating synergism *in vitro* resulting in a total reversal of platelet aggregation at concentrations which are ineffective when the drugs are applied by themselves.

Prolongation of the human life-span has brought a growing incidence of thromboembolic diseases in its wake. Beside the common systemic anticoagulants heparin and coumarines, inhibition of the primary phase of blood coagulation comprizing the adhesion, viscous metamorphosis, spreading reaction and aggregation of platelets, has been attracting increasing interest as a new avenue for antithrombotic prevention. One of the first drugs which have been examined under this aspect and found to inhibit platelet aggregation was acetylosalicylic acid [14, 15] although the inhibitory action of papaverine had been noted earlier [1]. There are data concerning the aggregation inhibiting property of spasmolytic dugs, tranquillizers, dextran, and pyrimido-pyrimidine derivatives [12]. However, concentrations which have been found inhibitory *in vitro*, *i.e.* 10^{-4}, 10^{-5} *M*, are not attained *in vivo* unless massive doses carrying untoward side-effects are administered. This gives particular significance to the search for new drugs acting as inhibitors at lower concentrations. Prostaglandins (PGs) were also found to affect blood clotting; its action is mainly directed at the primary phase of haemostasis although it also affects systemic haemostasis [2]. Emmons et al. [4] and Kloese [6] showed that it was a potent inhibitor of ADP-induced aggregation and glass adhesion of platelets both *in vivo* and *in vitro*, in humans as well as in rats. On the other hand, PGE_2 was found to enchance aggregation. The inhibitory effect of PGE_1, PGA_1 and $PGF_1\alpha$ is related to the rate of cAMP synthesis in the platelet membrane, induced by these compounds [7, 21] not only failed to confirm the potentiating effect of PGE_2 in platelet-rich human plasma (PRP) but found it even inhibitory to platelet aggregation. Chandra-Sekhar [3] tested eight prostaglandins E_1, E_2, A_1, A_2,

$F_1\alpha$, $F_1\beta$, $F_2\alpha$ and $F_2\beta$ for their effect on platelet aggregation induced by ADP, thrombin and collagen. All the PGs inhibited aggregation; PGE_1 was the most active. Administered in doses of 3 mg/kg to rats in intravenous infusion for 30 min it prolonged aggregation time, whereas doses of 0.1 μg/kg/min had no effect in humans [2]. An interesting fact emerging from the study of PGE_2 by BORN's photometric procedure was that at concentrations ranging from 0.1 to 3.0 μg/ml it accelerated platelet aggregation, at concentrations of 10 μg/ml it inhibited it. The accelerating mechanism of the substance failed, however, to take effect unless the concentrations of ADP were in the range of 0.5 to 1.0 μg/ml. In the experiments of CHANDRA-SEKHAR PGE_1 showed a potent thrombolytic effect and significantly prolonged coagulation time *in vitro* in humans, dogs, rats and rabbits. It also proved inhibitory to clot retraction.

WOLF and SHULMANN [19] studied the blood platelets for energy generation and release reaction. PGE_1 proved inhibitory to both, similarly to theophylline and cAMP. Inhibition of the release reaction required higher concentrations of PGE_1 (10^{-4} *M*) than did platelet aggregation (10^{-8} *M*). SHIO et al. [16] studied PGE_1 and PGE_2 by photometry for their joint effect on ADP-induced aggregation of rat platelets. They found PGE_1 in itself (13 μg/ml) to reverse, and PGE_2 (1 μg/ml) to accelerate platelet aggregation. When applied in combination, PGE_2 counteracted the inhibitory effect of PGE_1 on aggregation. The substances were also found to inhibit ADP-induced metamorphosis of rat platelets.

The various compounds affecting the aggregation of platelets have been subject of our studies for years. The compounds examined thus far *in vitro* and *in vivo* include spasmolytic drugs, tranquillizers and theophylline [9, 13, 5]. In the present study we have examined the prostaglandins PGE_1 and PGE_2 for their effect on platelet aggregation, alone and in combination with the spasmolytic drug dotaverine.

Material and method

Human platelet-rich plasma (PRP) was used. Aggregation was determined partly by measurement of aggregation time according to HOVIG [20], partly by the photometric procedure of BORN [1]. For details we refer to our earlier papers [5, 8]. For the production of aggregation, ADP solution was used at 0.7 μg/ml final concentration.

Results

Effect of PGE_1 on ADP-induced platelet aggregation

PGE_1 proved a highly potent inhibitor of ADP-induced platelet aggregation. Results are presented in Table I and Fig. 1, which shows a dose-response curve plotted from mean values. The inhibitory effect of PGE_1 on aggregation

appeared at a dilution of 10^{-8} *M*. At 10^{-7} to 10^{-6} it partially inhibited, at 1×10^{-5} *M* it significantly prolonged, and at 2×10^{-5} *M* it completely reversed ADP-induced platelet aggregation. Results of photometric measurements are seen in Fig. 2. Addition of PGE_1 at high concentrations to the mixture at the peak of aggregation resulted in a prompt and total disaggregation, as a sign of

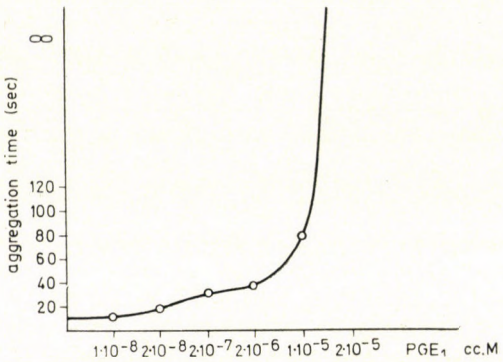

Fig. 1. Inhibitory effect of PGE_1 on ADP-induced platelet aggregation, as a function of concentration

Table I

Effect of prostaglandin E_1 on ADP-induced platelet aggregation in vitro

Number of cases	Aggregation time (sec)							
	Prostaglandin E_1 (*M*)							
	0.0	1×10^{-8}	2×10^{-8}	2×10^{-7}	2×10^{-6}	5×10^{-6}	1×10^{-5}	2×10^{-5}
1	13	14	18	26	33	45	55	—
2	14	14	16	21	30	61	120	—
3	14	15	17	28	51	59	65	—
4	10	11	14	17	28	34	60	—
5	12	12	16	21	50	53	80	—
Mean	13±1.7	13±1.7	16±1.5	23±4.4	38±11	51±11	76±26	—

the potent inhibitory effect. After incubation with PRP, PGE_1 was found to reverse ADP-induced platelet aggregation. In conformity with the former results, at 10^{-5} *M* it was compeletely, at 2×10^{-7} *M* partially, inhibitory.

Effect of PGE_2 on ADP-induced platelet aggregation

PGE_2 displayed a potent inhibitory effect.

In Table II and Fig. 3 it is seen that the inhibition of platelet aggregation appeared at a final concentration of 2×10^{-6}. Parallel with the concentra-

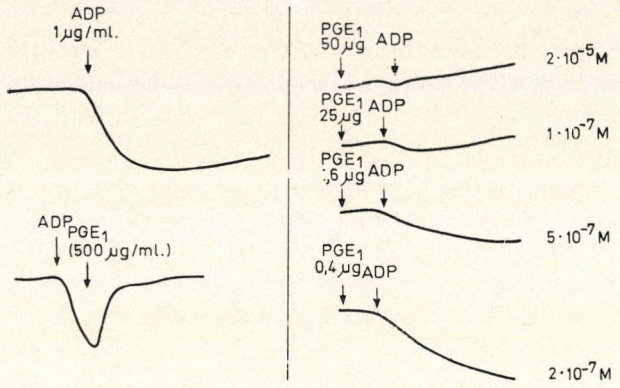

Fig. 2. Effect of PGE_1 on ADP-induced platelet aggregation

tion the inhibitory effect increased and at a final concentration of 1.2×10^{-4} *M*, PGE_2 caused a complete reversal of ADP-induced platelet aggregation in every case.

Table II

Effect of prostaglandin E_2 on ADP-induced platelet aggregation in vitro

Number of cases	Aggregation time (sec)					
	Prostaglandin E_2 (*M*)					
	0.0	2×10^{-6}	2×10^{-5}	4×10^{-5}	8×10^{-5}	1.2×10^{-4}
1	14	18	20	28	120	—
2	14	15	17	26	50	—
3	17	18	19	28	37	—
4	14	16	19	42	78	—
5	15	18	29	44	103	—
Mean	15±1.6	17±1.4	21±4.7	34±8.6	78±34	—

Fig. 4 presents the results of photometric measurements. Addition of PGE_2 at high concentration to the mixture at the peak of aggregation resulted, after a transitory acceleration of aggregation, in a disaggregation. On previous incubation with PRP, PGE_2 had only an inhibitory effect.

In conformity with other data, ADP-induced aggregation was inhibited 100 times more by PGE than by PGE_2.

Next, the effect of the two PGs was tested in combination with drotaverine, a spasmolytic drug which in earlier studies [5, 9, 13] was found to inhibit platelet aggregation, although less potently than papaverine. The inhibitory effect of drotaverine begins at 3.4×10^{-4} *M*, to cause total inhibition

at 8.5×10^{-4} *M*. Results are summarized in Table III. All the three drugs were applied at concentrations which fail significantly to affect aggregation time. When PGE_1 and drotaverine were added together to PRP, the doses inactive in themselves were found to prolong aggregation time significantly. In combi-

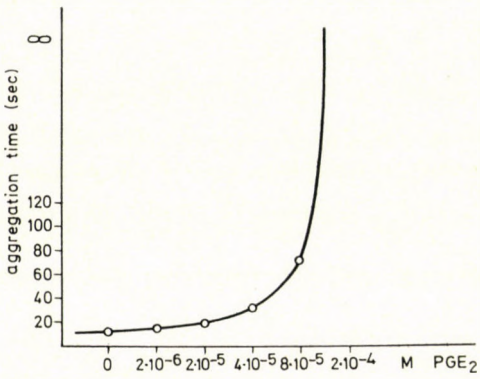

Fig. 3. Inhibitory effect of PGE_2 on ADP-induced platelet aggregation as a function of the concentration

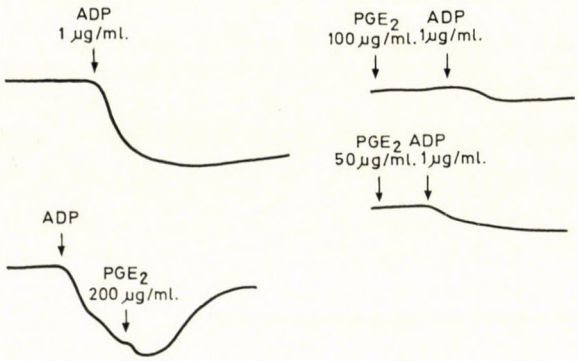

Fig. 4. Effect of PGE_2 *in vitro* on ADP-induced platelet aggregation

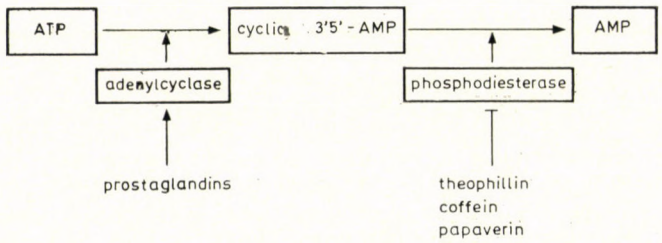

Fig. 5. Mechanism of the inhibitory effect of prostaglandins on platelet aggregation

Table III

Effects of PGE_1, PGE_2, drotaverine, PGE_1 + drotaverine, PGE_2 + drotaverine in different concentrations on ADP-induced platelet-aggregation

Number of cases	Concentration, *M*			Aggregation time (sec)				
	PGE_1 $\times 10^{-7}$ *M*	PGE_2 $\times 10^{-5}$ *M*	Drotaverine $\times 10^{-4}$ *M*	PGE_1	PGE_2	Drotaverine	PGE_1 + Drotaverine	PGE_2 + Drotaverine
1	0	0	0	12	12	12	12	12
2				13	13	13	13	13
3				11	11	11	11	11
1				25	15	12	70	21
2	1.0	1.0	1.5	23	14	13	53	16
3				21	13	11	37	15
1				75	21	13	no aggr.	80
2	2.0	2.0	3.0	34	19	13	no aggr.	38
3				26	18	13	no aggr.	28
1				105	40	17	no aggr.	no aggr.
2	10.0	4.0	6.0	82	45	15	no aggr.	no aggr
3				61	40	17	no aggr.	no aggr.

nation with drotaverine PGE_1 too proved a more potent inhibitor than PGE_2, the concentrations causing total inhibition having been 2×10^{-7} for PGE_1, and 4×10^{-5} for PGE_2.

Discussion

The results of the present study are consistent with the observation that among the PGs which represent the most potent inhibitors of platelet aggregation, PGE_1 has the strongest inhibitory effect. In respect of the mechanism of action of PGs, KLOESE showed that PGE_1, PGA_1 and $PGF_1\alpha$ enhanced the synthesis of cAMP in the platelet membrane, the extent of this synthesis being directly related to the inhibitory potency. It was indeed been demonstrated by MARQUIS et al. [11] that cAMP in itself inhibits platelet aggregation, and inferred from this that PGE_1 exerts its inhibitory effect by generating cAMP by activating adenyl cyclase. In fact, the level of cAMP is determined primarily by the activity of adenyl cyclase and phosphodiesterase, as it has been represented in Fig. 5. As regards PGE_2, SHIO et al. [16] demonstrated that the substance in itself does not affect the cAMP level of rat platelets, but reduces significantly the PGE_1-induced increase in the cAMP level which would seem to indicate that in rat platelets PGE_2 acts through an antago-

nism to intracellular PGE_1. As regards the effect of spasomolytics, papaverine was shown to inhibit the activity of phosphodiesterase [10] thus enhancing cAMP synthesis and inhibiting platelet aggregation, a mechanism which would seem to offer the best explanation for the synergism of the PGs and drotaverine. Study of this synergism might open new avenues for antithrombotic prevention.

REFERENCES

1. Born, G. V. R.: Nature (Lond.) **194,** 927 (1962).
2. Carlson, L. A., Irion, E., Orö, L.: Life Sci. **7,** 85 (1968).
3. Chandra-Sekhar, N.: J. med. Chem. **13,** 39 (1970).
4. Emmons, P. R., Hampton, J. R., Harrison, M. J. G., Honour, A. J., Mitchell, J. R. A.: Brit. med. J. **2,** 468 (1967).
5. Gregus, Z.: unpublished observations.
6. Kloese, J.: Influence of Prostaglandin on Platelet Adhesiveness and Platelet Aggregation In: Prostaglandins (S. Bergström, B. Samuelson, eds) p. 241. Interscience Publishers, London 1967.
7. Kloese, J.: Experientia (Basel) **26,** 307 (1970).
8. Losonczy, H., Majtényi, A., Nagy, I.: Prostaglandin Symposion, Budapest 1973.
9. Losonczy, H., Nagy, I., Gregus, Z.: The effect of spasmolytic drugs on ADP and collagen-induced platelet aggregation. International Symposium on drug-induced metabolic changes. (T. Javor and A. Gogl. eds.) Publishing House of the Hungarian Academy of Sciences, Budapest 1974.
10. Markwardt, F., Hoffmann, A.: Biochem. Pharmacol. **19,** 2519 (1970).
11. Marquis, N. R., Vigdahl, R. L., Tavormina, P. A.: Biochem. biophys. Res. Comm. **36,** 965 (1969).
12. Mustard, J. F., Packham, M. A.: Pharmacol. Rev. **22,** 97 (1970).
13. Nagy, I., Losonczy, H., Gregus, Z.: The effects of some spasmolytic drugs on platelet aggregation. IV. Abstr. Int. Congress of Thrombosis and Haemostasis. Vienna 1973, p. 372.
14. Quick, A. J.: Am. J. Med. Sci. **252,** 265 (1966).
15. Scharrer, I., Schepping, M., Breddin, K.: Klin. Wschr. **47,** 1318 (1969).
16. Shio, H., Ramwell, P. W., Jessup, S. J.: Prostaglandins **1,** 1 (1972).
17. Vighahl, R. L., Marguis, N. R., Tavormina, P. A.: Biochem. biophys. Res. Comm. **37,** 409 (1969).
18. Wolf, S. M., Shulmann, N. R.: Biochem. biophys. Res. Comm. **35,** 265 (1969).
19. Wolf, S. M., Shulmann, N. R.: Biochem. biophys. Res. Comm. **41,** 128 (1970).
20. Hovig, T.: Thrombos. Diath. Haemorrhag. *9,* 248 (1963).
21. Irion, E., Blombäck, M.: Scand. J. Clin. Lab. Invest. *24,* 141 (1969).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (3—4), pp 165—172 (1974)

COMPARTMENTALIZATION OF HAEMIC CELLS AND INTIMATE CONTACT BETWEEN ENDODERMAL EPITHELIUM AND HAEMOPOIETIC PRECURSOR CELLS IN HUMAN YOLK SAC

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Received March 14, 1974

Light microscopic investigation of two human yolk sac samples suggested that the haemopoietic cells of the early human embryo are compartmentalized, *i.e.*, early haemopoietic precursors which do not contain stainable haemoglobin are located near the endoderm, whereas embryonal erythroblasts with abundant eosinophilic cytoplasm are located mainly in vessels.

Some haemopoietic precursors were discovered in an endodermal cell-box. There is no evidence of either haemopoietic stem cells developing from the endoderm, or apparently enclosed precursors giving rise to circulating cells. The intimate contact between endodermal epithelium and haemopoietic precursor cells in human yolk sac awaits explanation. The existence of intercellular communication is likely.

Introduction

A 29 years old pregnant patient suffering from chronic idiopathic aplastic pancytopenia for more than 15 months was treated with intravenous injection of unprocessed yolk sac and liver material deriving from her own 22 mm crown-rump length embryo. Full clinical and subtotal haematological recovery occurred, without any direct evidence of a take of the injected material. Before the intervention the disease had been transfusion-dependent and failed to improve on conventional therapy. Neither blood transfusions nor any other treatment were necessary during recovery. The patient has become free of complaints and now, four years after the intervention is still in remission [10].

Owing to the small amount of injected yolk sac cells (mostly epithelial ones) the significance of these cells remains uncertain. Histological investigation of the human yolk sac suggested that the endodermal epithelial cells on the inner surface of the yolk sac are in an intimate contact with early haemopoietic progenitors.

In any event, the interesting fact that membranes with only epithelial components (so-called thymic Anlage) transplantated under the renal capsule of mice, induce the development of complete little thymuses, has been demonstrated by BIGGAR et al. [1].

Material

Fresh yolk sac samples have been investigated. They originated from two intact human embryos 16.0 and 19.0 mm in crown-rump length, respectively, delivered by hysterotomy for fibromyomatosis. Four to six micron sections were stained with conventional haematoxylin–eosin.

Results

Figures 1 to 4 serve to demonstrate the source of my suggestions according to which certain early haemopoietic cells of the human yolk sac (1) could be outstanding cells, (2) may be located in a "hole", and the nucleus of the endodermal host cell cannot be detected. The degree of incarceration of free haemic cells varies, but the apparently incarcerated cells appear to be uninjured in each case and even mitotic figures can be detected.

It would be hazardous to predict the frequency of this phenomenon on the basis of this small number of observations, but it was not difficult to discover it in serial diagonal sections (Table I).

Table I

Appearance of epithelial cells in pseudotubular folds of the yolk sac endoderm in a 16 mm crown-rump length human embryo

Endodermal epithelial cell:		
with its own nucleus	94/200 cells	~ 47%
with apparently empty cytoplasm	79/200	~ 40%
with haemopoietic precursor in the cytoplasm		
clearly demonstrable	16/200	~ 10%
probable	8/200	
with own nucleus and precursors	2/200	
with two precursors in the cytoplasm	1/200	
with mitotic precursor	1/200	

In the sections the haemopoietic cells of the embryo were for the most part compartmentalized. Early haemopoietic cells lacking stainable haemoglobin are located near the endoderm and form occasional clusters (Fig. 5), whereas embryonal erythroblasts with abundant eosinophilic cytoplasm are located mainly in vessels (Fig. 6). A mixing of the two compartments was not apparent. "Embryonic" erythroblasts were not surrounded by endodermal epithelial cells, and there was no morphological evidence to support the assumption that embryonic erythroblasts with abundant eosinophilic cytoplasm derive from the early precursor cells shown in Figures 1 to 5.

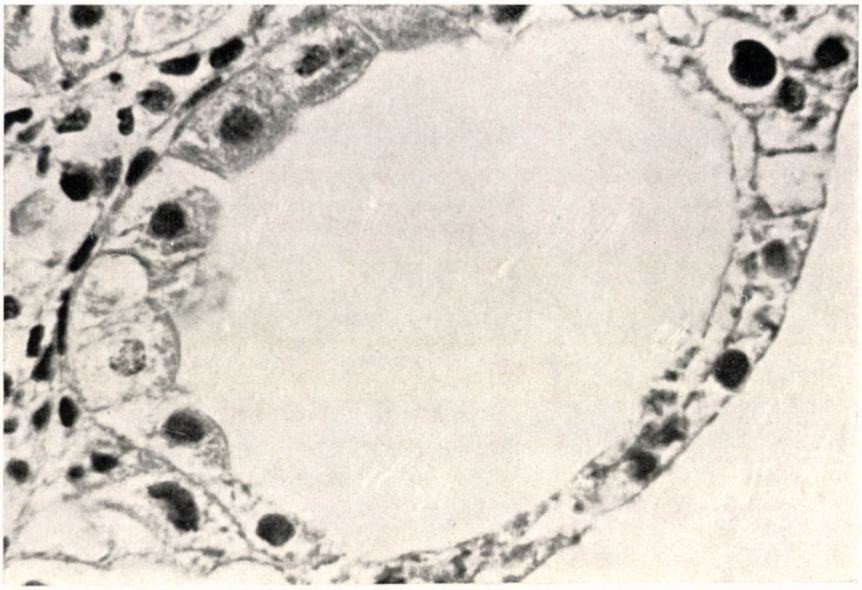

Fig. 1. Yolk sac endodermal pseudotubule: 16 mm CR-length human embryo. Apparent stages of nuclear demarcation with a fully demarcated nucleus at the right upper angle. The apparently incarcerated nucleus is 8—10 μ in diameter. Fixed in formalin and stained with haematoxylin–eosin

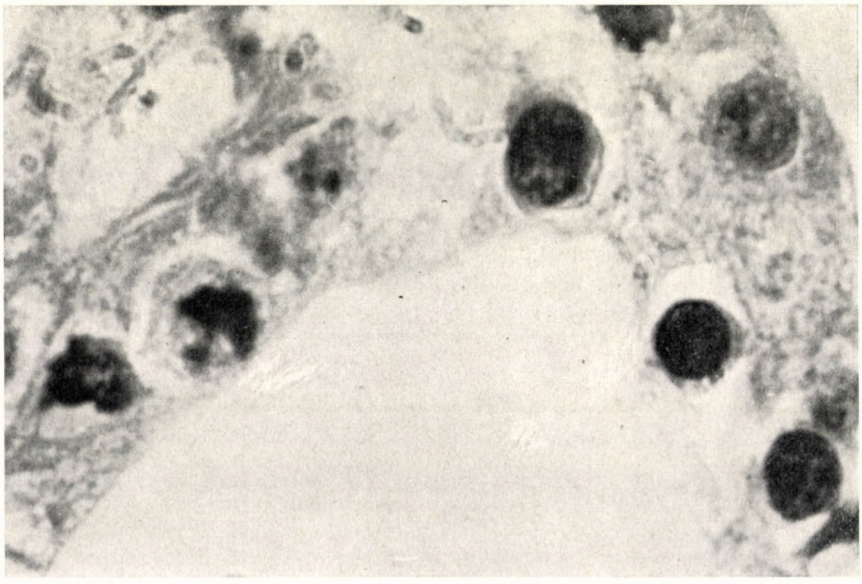

Fig. 2. Yolk sac endodermal pseudotubule: 16 mm CR-length human embryo. The cytoplasm of the boxed cells is well visible. The incarcerated nuclei measure 8—10 μ in diameter. A mitotic figure is shown. Haematoxylin–eosin

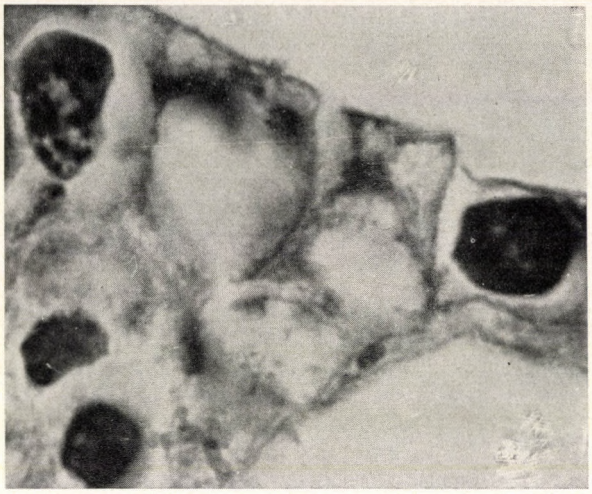

Fig. 3. Yolk sac endodermal cells: 16 mm CR-length human embryo. Two intracytoplasmic early haemopoietic precursor cells 15—20 μ in diameter with deep basophilic cytoplasm at the upper part, and a presumed stem cell at the bottom. Haematoxylin–eosin

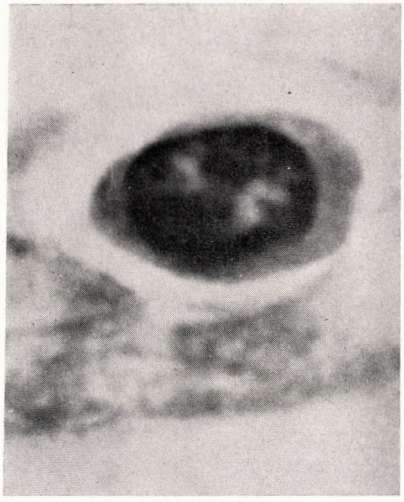

Fig. 4. Yolk sac endodermal pseudotubule: 16 mm CR-length human embryo. An intracytoplasmic early haemopoietic precursor cell resembling proerythroblast at high magnification. The longer diameter of the boxed cell measures 21 μ. Haematoxylin–eosin

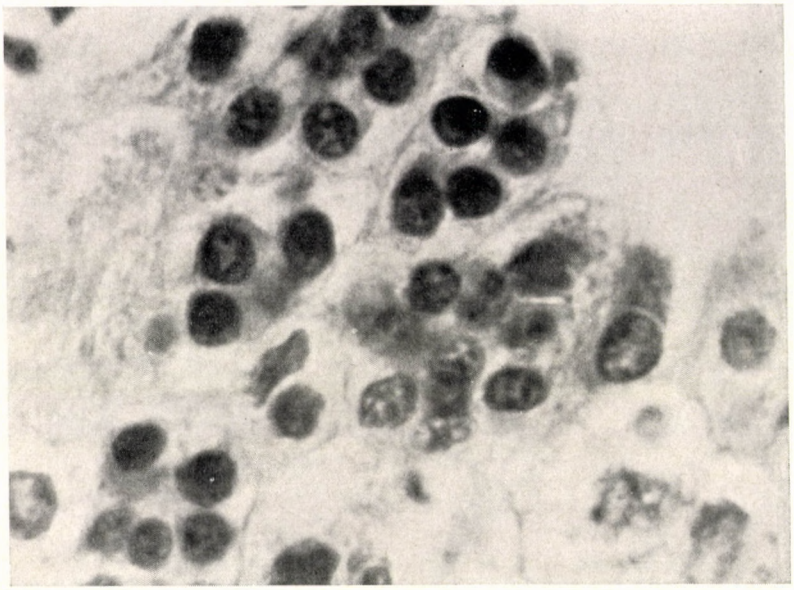

Fig. 5. Yolk sac endoderm: 16 mm CR-length human embryo. Small haemopoietic precursor cells 8—10 μ in diameter with light basophilic, narrow cytoplasm forming at least five clusters. Apparent demarcation of a seemingly similar cell at middle right margin. Haematoxylin–eosin

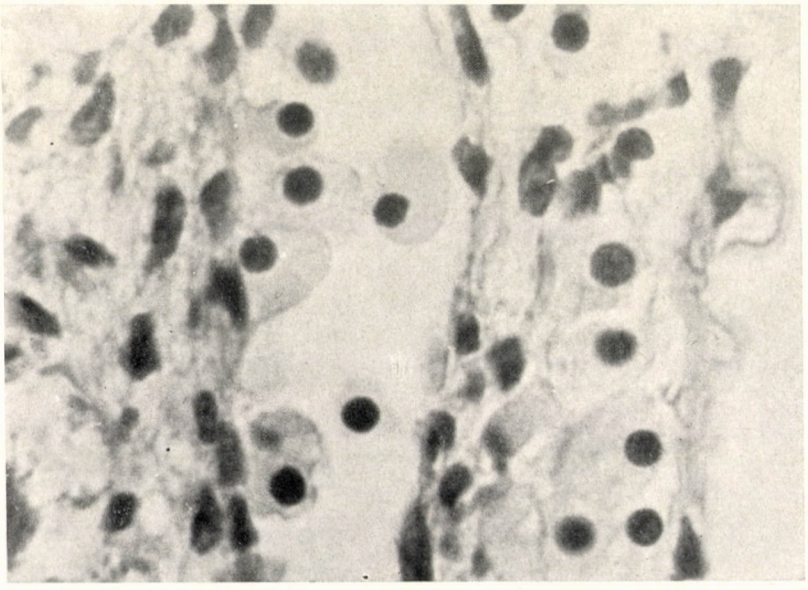

Fig. 6. Yolk sac of 16 mm CR-length human embryo, with embryonic erythroblasts having abundant eosinophilic cytoplasm in a vessel (middle) and in the extravascular mesoderm (right). The longer axis of embryonic erythroblasts ranges from 12 to 26 μ. The mesothelium is seen at the right margin, whereas the clear area at the left lower border belongs to "empty" endodermal cells. Haematoxylin–eosin

Discussion

At first, the presence of an occasional haemopoietic precursor cell in the cytoplasm of an endodermal cell was considered an artifact. The lack of an own nucleus in these cells spoke, however, against this possibility, so that phagocytosis or emperipolesis have not been taken into account. Cells such as the one at the right upper corner of Fig. 2, especially spoke against their being artifacts.

The occurrence of haemocytoblasts in endodermal epithelial cells has already been described by BLOOM and BARTELMEZ in a 10.2 mm CR-length human embryo. Even mitosis of an intra-endodermal haemocytoblast has been noted in a 12 mm CR-length embryo. A similar process was described by SAXER in the yolk sac endoderm of sheep, cat, and pig embryos as early as 1896. Nevertheless, the cautious notes of BLOOM and BARTELMEZ should be respected before we tend to suggest that endodermal epithelial cells have a part in embryonal haemopoiesis.

Our photographs suggest that at the stage of human yolk sac development reported in this paper the endodermal epithelium appears in at least three forms. There are cells with (1) preserved nuclei; (2) empty cytoplasm and (3) demarcated haemopoietic precursors apparently incarcerated into the cytoplasm. The origin of the last cells remains a matter of speculation. No pathway is known to explain how these precursor cells reach the circulation after their eventual release. Although these cells could be discarded ones, their apparently intact condition speaks against this possibility.

Although an unorthodox point of view, earlier (cf. GLADSTONE and HAMILTON, [6] as well as new data including ultrastructural studies [9], occasionally suggested that the endoderm is the site of origin of the blood cells in the human yolk sac. On the other hand, electron microscopic studies of HESSELDAHL and LARSEN [8] revealed no haemopoietic foci in the endoderm in any of 21 human yolk sac specimens. Either these authors, or FUKUDA concluded that contact of erythroid cells with the endodermal epithelium seems to be necessary for their differentiation. Owing to the villous structure of the yolk sac one must consider that even cell clusters, such as represented in Fig. 6, could exhibit mesenchymal cells located just below the endodermal layer. In any event, apparently enclosed haemopoietic precursors, *i.e.* close appositions to, and pronounced interdigitations with, endodermal epithelial cells, have been discovered in the course of our present electron microscopic study performed together with Dr. I. Balogh and even an abundance of desmosomal junctions could be discovered. In this sense, the existence of *intercellular communication* is very likely.

The haemopoietic role of the animal yolk sac has clearly been demonstrated by MOORE and METCALF [13]. METCALF and MOORE also reviewed the

literature on the origin of haemopoietic cells of the embryonic liver [12]. An endodermal origin of human hepatic haemocytoblasts was suggested by, *e.g.*, THOMAS et al. [16] and THOMAS and YOFFEY [17]. Compartmentalization of haemopoietic cells in the human embryonic liver has been recently emphasized by FUKUDA [15]. Investigation of murine yolk sac samples by RIFKIND et al. [14] gave rise to similar conclusions. The last two studies suggest that haemopoietic stem cells derive from mesenchymal cells of the septi transversi.

Lymphoid representation is absent in the early developmental stage, and the cells which resemble lymphocytes under the light microscope cannot be identified as lymphocytes [3, 7]. Despite this unanimously accepted concept, on the base of both morphological [11] and functional observations, we now investigate the possible prebursal capacity of the lymphocyte-like cells which we have found in the early foetal liver as well as in the yolk sac.

Acknowledgements

The cooperation of Dr. Cs. KISS and Dr. Éva MAGYAR (Postgraduate Medical School, Budapest) and Dr. E. PUSKÁS and the Staff of the Second Department of Obstetrics and Gynaecology, Semmelweis University Medical School, Budapest, as well as of Dr. GY. PETRÁNYI jr. of the First Department of Medicine, Semmelweis University Medical School, Budapest is acknowledged with thanks. Ultrastructural studies carried out with Dr. I. BALOGH of the Institute of Forensic Medicine, Semmelweis University Medical School, Budapest, now in progress, appear to resolve some of our problems regarding both yolk sac and liver haemopoiesis in the early human embryo.

REFERENCES

1. BIGGAR, W. D., STUTMAN, O., GOOD, R. A.: Morphological and functional studies of fetal thymus transplants in mice J. exp. Med. **135,** 793—807 (1972).
2. BLOOM, W., BARTELMEZ, G. W.: Hematopoiesis in young human embryos. Amer. J. Anat. **67,** 21—52 (1940).
3. DICKE, K. A., VAN NOORD, M. J., MAAT, B., SCHAEFER, U. W., VAN BEKKUM, D. W.: Identification of cells in primate bone marrow resembling the hemopoetic stem cells in the mouse. Blood **42,** 195—208 (1973).
4. FUKUDA, T.: Fetal hemopoiesis I. Electron microscopic studies of human yolk sac hemopoiesis. Virchows Arch. exp. Path. Abt. B. **14,** 197—213 (1973a).
5. FUKUDA, T.: Undifferentiated mononuclear cell in human embryonic liver: presumptive hemopoietic stem cell. Virchows Arch. exp. Path. Abt. B. **14,** 31—34 (1973b).
6. GLADSTONE, J. R., HAMILTON, W. J.: A presumite human embryo (Shaw) with primitive streak and chorda canal, with special reference to the development of the vascular system. J. Anat. (Lond.) **76,** 9—44 (1941).
7. HAAS, R. J., BOHNE, F., FLIEDNER, T. M.: Cytokinetic analysis of slowly proliferating bone marrow cells during recovery from radiation injury. Cell Tissue Kinet. **4,** 31—45 (1971).
8. HESSELDAHL, H., LARSEN, J. F.: Hemopoiesis and blood vessels in human yolk sac: an electron microscopic study. Acta anat. (Basel) **78,** 274—294 (1971).
9. HOYES, A. D.: The human foetal yolk sac. An ultrastructural study of four specimens. Z. Zellforsch. **99,** 469—490 (1969).
10. KELEMEN, E.: Recovery from chronic idiopathic bone marrow aplasia of a young mother after intravenous injection of unprocessed cells from the liver and yolk sac of her 22 mm CR-length embryo. Scand. J. Haemat. **10,** 305—308 (1973).

11. KELEMEN, E.: Light microscopy of scattered, unprocessed haemopoeitc precursor cells in liver smears of a 6.35 mm crown-rump length human embryo. Exp. Hematol. **2,** (1974).
12. METCALF, D., MOORE, M. A. S.: *Haemopoietic Cells*, North Holland, Amsterdam 1971.
13. MOORE, M. A. S., METCALF, D.: Ontogeny of the hemopoietic system. Yolk sac origin of in vivo and in vitro colony forming cell in the developing mouse embryo. Brit. J. Haemat. **18,** 279—296 (1970).
14. RIFKIND, R. A., CHUI, D. EPLER, H.: An ultrastructural study of early morphogenetic events during the establishment of fetal hepatic erythropoiesis. J. Cell Biol. **40,** 343—365 (1969).
15. SAXER, F.: Über die Entwicklung und den Bau der normalen Lymphdrüsen und die Entstehung der roten und weissen Blutkörperchen. Anat. Hefte, Abt. I., **6,** 347—352 und Tafel XV—XXII (1896).
16. THOMAS, D. B., RUSSEL, P. M., YOFFEY, J. M.: Pattern of hemopoiesis in foetal liver. Nature (Lond.) **187,** 876—877 (1960).
17. THOMAS, D. B., YOFFEY, J. M.: Human foetal haematopoiesis II. Hepatic haematopoiesis in the human foetus. Brit. J. Haemat. **10,** 193—197 (1964).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (3—4), pp 173—179 (1974)

THE AUTOIMMUNE STATUS IN GRAVES' DISEASE

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Received March 22, 1974

Laboratory studies revealed in 175 patients with Graves' disease subjected to radioiodine treatment and in 17 recent untreated cases of Graves' disease, as compared to 44 normal control subjects, that Graves' disease is often associated with other autoimmune disease; in Graves' disease the antithyroid, antimuscle and antikidney antibody titres and the antinuclear reaction are significantly increased; a significant correlation was found between the antithyroid antibody level, the antinuclear reaction and the duration of the disease. The titres increased during the first 7 to 8 years and declined significantly after 15 years.

The observation that allergic processes are adversely affected by hyperthyroidism dates back to the forties. Demonstration of Lats by Adams and Purves [1] gave new impetus to the studies of these relationships, particularly when the immunoglobulin nature of the hormone was demonstrated by Kriss et al. [7], Miyai et al. [8] and others, and when its antibodylike attachment to the microsomal fraction of the thyroid had been proved by Beall and Solomon [2]. Insight into the activity of this hormone resulted in a growing tendency to regard Graves' disease as an autoimmune disease. More recent evidence has, however, cast some doubts on this view. The findings published by Sellers et al. [11], Chopra et al. [3], Wong and Doe [13] and others have questioned the aetiological role of Lats. Recently, the tendency to incriminate cell-borne autoimmune reactions and stimulation instead of the role of Lats has gained prevalence [12]. The Lats-period has been none the less fruitful, inasmuch as it has stimulated research into the autoimmune relationships of Graves' disease, and thus brought various interesting facts to light. It has been demonstrated by these observations that Graves' disease carries a high incidence of (excessively) pathologically increased antithyroid antibody levels, second only to Hashimoto's disease in this respect [9]. It has been also confirmed on clinical grounds that association of hyperthyroidism with pernicious anaemia, primary adrenocortical insufficiency, myasthenia gravis, hyperplasia of thymus, is common [10].

The hyperthyroid patients of our Department having had radioiodine treatment between 1957 and 1970, have systematically been followed up. These studies, concerned primarily with the restitution of thyroid suppressibility,

have been extended to a survey of anamnestic and katamnestic nature and to laboratory investigations. We are reporting here the results of some tests so as to substantiate and to supplement the foregoing observations.

Material and method

A group of 175 patients suffering from Graves' disease who had had radioiodine treatment was studied. A detailed history was taken in every case. The clinical thyroid status was evaluated by the statistical method of CROOKS et al. [4]. The laboratory investigations included thyroid ^{131}I-retention, suppression test according to WERNER and SPOONER [14], the HAMOLSKY-test or some of its modifications (T_3), measurements of T_4, serum cholesterol, and in doubtful cases, of serum PBI.

Information concerning the immune status was obtained on the grounds of the LE-cell phenomenon, antibodies to thyroid, kidney and muscle homogenisates by passive haemagglutination tests. Titres of 1/16 and in excess of it were regarded as positive. The titres of antinuclear and antiglobulin antibodies (Waaler-Rose reaction) were also determined. Obstacles of technical nature prevented us from performing the full range of investigations in every case of the series.

For control purposes, 44 normal blood donors were tested for antiorgan and antinuclear antibodies, 48 other normal blood donors, for the Waaler-Rose reaction, simultaneously with the patients.

Moreover, 17 patients with recent untreated Graves' disease were subjected to the nvestigations referred to above.

Results and evaluation

Of the 175 patients with Graves' disease having had radioiodine treatment, 156 were euthyroid, and 19 moderately hypothyroid. Patients with signs of recurrent thyroid hyperfunction were excluded from the study.

The history revealed autoimmune processes other than those involving the thyroid in 32 cases (18%), recurrent or severe tonsillitis in 77 cases (44%). Radioiodine treatment had been followed by some autoimmune syndrome of major gravity in 7 cases (4%).

Laboratory findings. The LE-cell phenomenon was positive in 2 cases. In 47 cases, only rosette formation was demonstrable.

Table I shows the titres of the antiorgan antibodies, the antinuclear reaction and the Rose reaction. With the only exception of the Rose test, the antibody titres were higher in the patients than in the control group. Statistical analysis with the four-tailed chi-square test revealed a significant difference ($p < 0.01$) in the antithyroid, antikidney, antimuscle and antinuclear antibody titres between the treated patients and the controls, the dilution 1/16 being taken as the orderline value. On the other hand, the Rose test showed no significant difference (0.9 p 0.8) between the two groups.

Next, the correlations between the antibody titres and certain clinical parameters were studied. According to HACKENBERGER et al. [5], thyroid suppressibility is associated with low, its non-suppressibility with high, anti-

Table I

Distribution of antibody-titres in radioiodine-treated patients with Graves' disease

Dilution	Antithyroid antibody		Antinuclear reaction (ANR)		Antimuscle antibody		Antikidney antibody		Rose test	
	treated group, Graves' disease	control group	treated group, Graves' disease	control group	treated group, Graves' disease	control group	treated group, Graves' disease	control group	treated group, Graves' disease	control group
2	18	3	21	14	26	18	19	29	15	—
4	44	31	49	19	47	21	61	10	32	10
8	43	10	55	10	40	5	42	4	53	15
16	36	—	34	1	17	—	16	1	36	14
32	17	—	6	—	12	—	6	—	20	6
64	7	—	1	—	1	—	3	—	9	2
128	3	—	1	—	—	—	1	—	—	1
256	—	—	—	—	1	—	—	—	2	—
Total	168	44	167	44	144	44	148	44	167	48

thyroid antibody titres. It must, however, be noted that these findings had been derived from methimazole-treated cases.

Table II shows the antibody titres in suppressible and in non-suppressible cases. They showed no significant difference. After log-transformation of the antibody titres of the individual patients yielding a normal distribution, the two-sample t test failed to reveal any significant difference between the two groups.

Table II

Distribution of antibody titres in radioiodine-treated suppressible and non-suppressible cases of Graves' disease

Dilution	Antithyroid antibody		Antinuclear reaction		Antimuscle antibody		Antikidney antibody	
	supp.	non-supp.	supp.	non-supp.	supp.	non-supp.	supp.	non-supp.
2	7	6	6	10	14	10	11	5
4	27	17	29	17	29	17	35	25
8	23	17	34	19	25	14	23	17
16	25	11	22	12	12	3	11	5
32	10	6	3	2	4	7	4	2
64	1	4	—	1	—	1	—	1
128	2	1	1	—	—	—	1	—
256	—	—	—	—	1	—	—	—
Total	95	62	95	61	85	52	85	55

Table III

Distribution of antinuclear antibody levels in relation to the length of the history

II \ I	2	4	8	16	32	64	128	256	Total number of tests
1—2	2	3	6	8	1				20
3—4	4	9	2	7					22
5—6	3	12	9	4	1	1			30
7—8	4	7	8	4	1		1		25
9—10	2	3	10	3	3				21
11—12	1	5	8	4					18
13—14		2	6	5					13
15	5	8	6						19

I Dilution
II Time (years) from onset of disease

To study the relationship of the duration of the disease and the antibody titres, the patients were divided into eight groups in an ascending order. In seven groups, the duration of disease increased within the range of 15 years by two years in each successive group, in the eighth group the disease had begun more than 15 years before. Tables III—VI illustrate the antibody levels in the different groups. Autoimmune activity displayed wide variations, with the peak falling between the 7th and 8th year after onset and the levels tending to decline after the 15th year. The variations in the titres of individual antibodies seemed at first congruent. Statistical analysis by log-transformation

Table IV

Distribution of antithyroid antibody titres in relation to the length of the history

II \ I	2	4	8	16	32	64	128	256	Total number of tests
1—2		4	6	8	1		1		20
3—4	6	4	6	2	2	1			21
5—6	5	7	8	7		3			30
7—8	1	6	6	5	4	1	2		25
9—10		5	7	5	4				21
11—12	1	6	5	3	2	1			18
13—14		3	2	5	3				13
15	4	9	3	1	1	1			19

I Dilution
II Time (years) from onset of disease

Table V

Distribution of antimuscle antibody titres in relation to the length of the history

I / II	2	4	8	16	32	64	128	256	Total number of tests
1—2	3	5	6		3				17
3—4	9	2	6	2		1			20
5—6	5	11	6	2	2				26
7—8	3	5	7	5	1				21
9—10	1	4	8	3	2				18
11—12	2	4	6	1	2				15
13—14	1	5	1	2	2				11
15	2	10	2	2					16

I Dilution
II Time (years) from onset of disease

revealed, however, certain differences. Analysis of variance between the individual groups yielded a significant difference in the levels of antinuclear antibody ($p < 0.05$) and of antithyroid antibody ($p < 0.025$). Moreover, in the group where the disease had been in existence for more than 15 years the values were significantly lower than for the cases of shorter duration. In contrast to the antinuclear and antithyroid antibodies, the antimuscle and antikidney antibodies in the individual groups revealed no significant correlation with the length of the history. The antinuclear and antithyroid antibody

Table VI

Distribution of antikidney antibody titres in relation to the length of the history

I / II	2	4	8	16	32	64	128	256	Total number of test
1—2	2	4	10	3					19
3—4	3	11	2	3					19
5—6	5	10	9	2	1				27
7—8	2	7	8	3		1	1		22
9—10	2	7	5	1	1				16
11—12	2	5	4	2	2				15
13—14		5	2	1					8
15	3	11	2	2					18

I Dilution
II Time (years) from onset of disease

levels (y-values) showed a significant *non-linear* correlation with the duration of the disease (x-value):

$$e_{xy} = \sqrt{\frac{q_x}{q_y}}$$

The values of recent, untreated cases of Graves' disease are seen in Table VII together with the control values. After log-transformation of the figures, the two-sample t and d tests revealed significantly higher mean values in the test group than in the controls.

Table VII

Distribution of antibody titres in normal controls and untreated patients with Graves' disease

Dilution	Antithyroid antibody		Antinuclear reaction (ANR)		Antimuscle antibody		Antikidney antibody		Rose test	
	Graves' disease	control group	Graves' disease	control group	Graves' disease	control group	Graves' disease	control group	Graves' disease	control group
2	4	3	5	14	3	18	5	29	3	—
4	5	31	2	19	5	21	5	10	1	10
8	5	10	7	10	4	5	4	4	6	15
16	2	—	2	1	2	—	2	1	3	14
32	1	—	1	—	1	—	1	—	1	6
64	—	—	—	—	—	—	—	—	1	2
128	—	—	—	—	—	—	—	—	—	1
256	—	—	—	—	—	—	—	—	—	—
Total	17	44	17	44	15	44	17	44	15	48

Our results have confirmed that in Graves' disease the antithyroid antibody titres persist at high levels long years after onset of the disease. In addition, the antinuclear, antimuscle and antikidney antibodies are increased in the sera of these patients, and they remain high even when thyroid hyperfunction has been controlled by radioiodine treatment. Their minor variations might be related to a periodicity in the activity of autoimmune processes. The significant decline in the antinuclear and antithyroid antibody titres after the 15th year raises the possibility that the autoimmune activity associated with Graves' disease tends to burn out in the course of time.

The finding increase in antibody activity in recent untreated cases seems to indicate that the changes observed in the treated patients were unrelated to radioiodine treatment, although this needs confirmation by further studies. An exception is the antithyroid antibody which persists at high values for long years also after subtotal thyroidectomy [6].

The present findings and the frequency of autoimmune conditions preceding the onset of Graves' disease or associated with it later, suggest the occurrence of an enchanced autoimmune reactivity in Graves' disease.

REFERENCES

1. Adams, D. D., Purves, H. D.: Endocrinology, **57,** 17 (1955).
2. Beall, G. N., Solomon, D. H.: Proc. roy. Soc. Med. **61,** 1302 (1968).
3. Chopra, I. J., Solomon, D. H., Johnson, D. E.: Metabolism **19,** 760 (1970).
4. Crooks, J., Murray, I. P., Wayne, E. J.: Quart. J. Med. **28,** 211 (1959).
5. Hackenberger, K., Schneider, K. R., Reinwein, D.: Dtsch. med. Wschr. **97,** 1264 (1972).
6. Hornung, G., Kanzler, G., Kuwer, E., Rausch-Stroomann, J. G., Reichel, K., Ströt-ges, W.: Dtsch. med. Wschr. **95,** 568 (1970).
7. Kriss, J. P., Pleshakov, V., Chein, J. R.: J. clin. Endocr. **24,** 1005 (1964).
8. Miyai, K., Fukuchi, M., Kumahara, Y. Abe, H.: J. clin. Endocr. **27,** 855 (1967).
9. Müller, W.: Ärztl. Fortbild. **18,** 161 (1970).
10. Müller, W.: Internist **11,** 17 (1970).
11. Sellers, E. A., Awad, A. G., Schönbaum, E.: Lancet **2,** 335 (1970).
12. Volpé, R., Edmonds, M., Lanki, L., Clarke, P. v. Rew, V. V.: Mayo Clin. Proc. **47,** 824 (1972).
13. Wong, E. T., Doe, R. P.: Ann intern. Med. **76,** 77 (1972).
14. Werner, S. C., Spooner, M.: Bull. N. Y. Acad. Med. **31,** 57 (1956).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (3—4), pp 181—186 (1974)

COMPARATIVE INTRACARDIAC AND MECHANOGRAPHIC STUDIES IN THE DOG

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Received March 28, 1974

The data derived from direct procedures (right and left ventricular, aortic and pulmonary pressure curves) and from indirect tracings (right and left apex cardiograms) in the dog have been examined with reference to the different phases of the heart cycle, in particular to the pre-ejection and ejection periods.

There was no essential difference between the data obtained by direct and by indirect procedures.

The apex cardiogram starts earlier than the corresponding ventricular pressure curve.

The right and the left apex cardiograms are different in time course.

Point E of the apex cardiogram seems to define the beginning of ejection.

There are few data on measurements of right ventricular function, in particular of the length of the heart cycle. Present knowledge is still based on the studies of WIGGERS [9] and RUSHMER [7].

LANDIS et al. [4] and BURCHELL and VISSCHER [3] studied the question in the isolated heart by means of high-frequency cinematograms, and ANZOLA [1] with implanted transducers in the closed-chest dog.

In the present experiments, synchronous direct recording of pressure curves and of mechanical tracings were made in dog with the aim of ascertaining whether the curves regarded as the right and the apex cardiogram resulted from the function of the respective ventricular wall. A further aim was to examine the relationships between mechanical tracings and simultaneous direct pressure curves.

Material and method

In this study, 18 mongrel dogs weighing 11 to 22 kg were used. Under Brevinarcon® anaesthesia and subsequent curarization transverse thoracotomy was performed and the pericardium was opened. Pressures in the ventricles and in the large vessels were measured with a Statham electromanometer through a thin polythene catheter introduced *via* the right or the left auricular appendage into the ventricles and subsequently into the large vessels. Pulsation of the right and left ventricle and the phonocardiogram were recorded by piezoelectric recorders and phonocardiographic microphones placed on the surface of the ventricles. The pressure curves, mechanical tracings, phonocardiograms as well as the standard *ECG* leads were registered by an 8 channel Hellige multiscriptor apparatus (Figs 1, 2).

Since the study was concerned with physiological relationships between the indirect (ext) and the direct (int) tracings, technically imperfect or grossly abnormal tracings were neglected. Thus the data presented were obtained from 14 right-heart and from 9 left-heart recordings including 6 simultaneous ones.

In view of the conflicting data in the literature, the first task was to find a starting point representing the beginning of ejection on the mechanical tracing. It was essential to ascertain whether the peak of the right apex cardiogram was suitable for the definition of the beginning of ejection, this being the only possibility for an indirect measurement of right ventricular ejection.

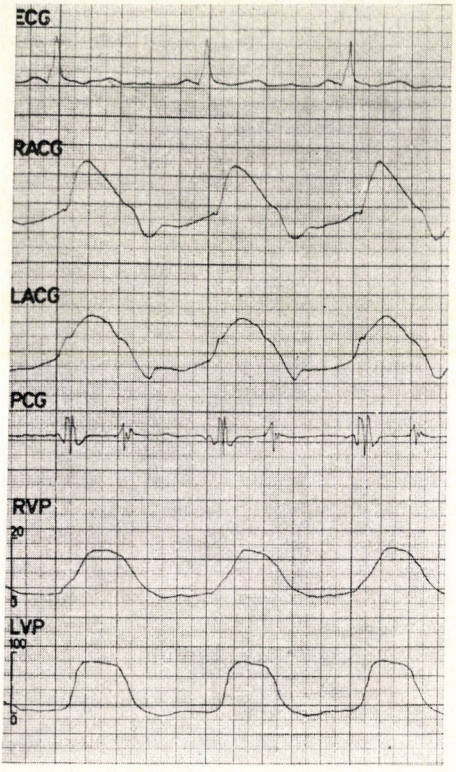

Fig. 1. Synchronously recorded electrocardiogram *(ECG)*, right apex cardiogram *(RACG)*, left apex cardiogram *(LACG)*, phonocardiogram *(PCG)*, right ventricular pressure *(RVP)*, left ventricular pressure *(LVP)*. Paper speed, 100 mm/sec

The parameters measures were, *pre-ejection period* (PEP_{ext}), obtained by measuring the distance between the *Q*-deflection of the ECG and the peak of the apex cardiogram, point E; *ejection period* (VET_{ext}), obtained by measurement of the distance between the peak of the apex cardiogram *(point E)*, and the aortic or pulmonary component of the second heart sound; the PEP_1*-period*, *i.e.* the distance between the *Q*-deflection of the *ECG* and the bottom of the apex cardiogram, a period representing the actual electromechanical interval; the PEP_2*-period*, *i.e.* the distance between the bottom and the peak of the apex cardiogram. All these time intervals were determined for the left as well as for the right ventricle. In addition, the following parameters were measured: the distance between *Q* and the bottom of the ventricular pressure curve for the left as well as for the right ventricle; the distances between *Q* and the respective

bottom of the aortic and the pulmonary pressure curves *(PEP$_{int}$)*. The distances between the respective bottom of the aortic and pulmonary pressure curves and the incisure were also determined *(VET$_{int}$)*. The measurements included the distance between the *Q* and the *O* point of the apex cardiogram (Table I).

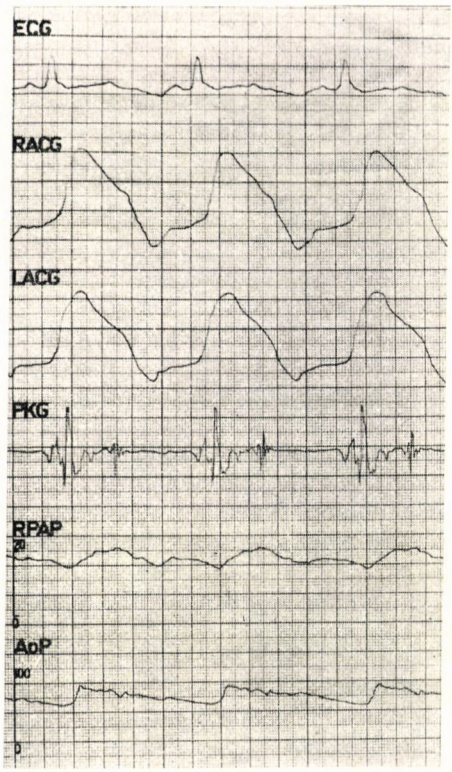

Fig. 2. Synchronously recorded electrocardiogram *(ECG)*, right apex cardiogram *(RACG)*, left apex cardiogram *(LACG)*, phonocardiogram *(PCG)*, right pulmonary arterial pressure *(RPAP)*, aortic pressure *(AoP)*. Paper speed, 100 mm/sec

Results

The differences between the values obtained by direct (int) and by indirect (ext) measurements are presented in Fig. 3. The differences were variable, but mostly of the same direction. The *pre-ejection period (PEP)* was slightly longer than expected on the basis of data in the literature where *PEP* values for the right ventricle obtained by cardiac catheterization are given in the range of 100 to 110 sec, whereas our mean value was 117.8 sec. On the other hand, from our findings that was clear that right ventricular ejection begins earlier than left ventricular ejection. Furthermore, the bottom of the cardiograms, in other words the starting point of the mechanical phenom-

ena, appeared earlier than did the increase in intraventricular pressure. This was at variance with the results of the closed-chest experiments by ANZOLA [1] according to which the mechanical tracings and the pressure curves started at the same point of time at the beginning of contraction. On the other hand, we found that while the left ventricular mechanical phenomena started 25 msec after the *Q*-deflection, the interval between the *Q* and the beginning of the rise in intraventricular pressure amounted to 59 msec. In the right ventricle, the

Table I

	Right ventricle	Left ventricle
Heart cycle ms	382.4	394.4
	±50.9	±46.2
PEP_1 ms	32.5	25.6
	±8.2	±5.7
PEP_2 ms	94.6	103.4
	±19.2	±18.3
PEP ext. ms	129.4	136.7
	±15.1	±13.2
PEP int. ms	117.8	126.7
	±11.9	±10.1
VET ext. ms	143.6	124.4
	±24.6	±16.7
VET int. ms	154.3	137.8
	±21.6	±13.8
Q—"O" ms	310.8	320.0
	±51.6	±42.6

mechanical phenomena started 32.5 msec after the *Q*-deflection, whereas the interval between *Q* and the bottom of intracardial pressure curve was 57.7 msec.

Next, we examined the relationship between the contraction time, the electromechanical interval and the period of increasing pressure of the two ventricles. Views are divided concerning the beginning of contraction as well as on the duration of ejection. It is generally thought that contraction of the left ventricle starts earlier. On the other hand, while some authors found that the duration of ejection was longer from the right than from the left ventricle some others observed the contrary. According to BRAUNWALD et al. [2],

contraction of the left ventricle started 19 msec earlier than that of the right but right ventricular ejection exceeded the left one by 50 msec. SOULIÉ [8] found that contraction of the left ventricle preceded that of the right by 30 msec and that its duration was 42 msec longer. On the other hand, RUSHMER [7] observed that the the pre-ejection period was shorter for the right than for the left ventricle, in other words, the interval between Q and the beginning of ejection was longer in the left than in the right ventricle. No data could be

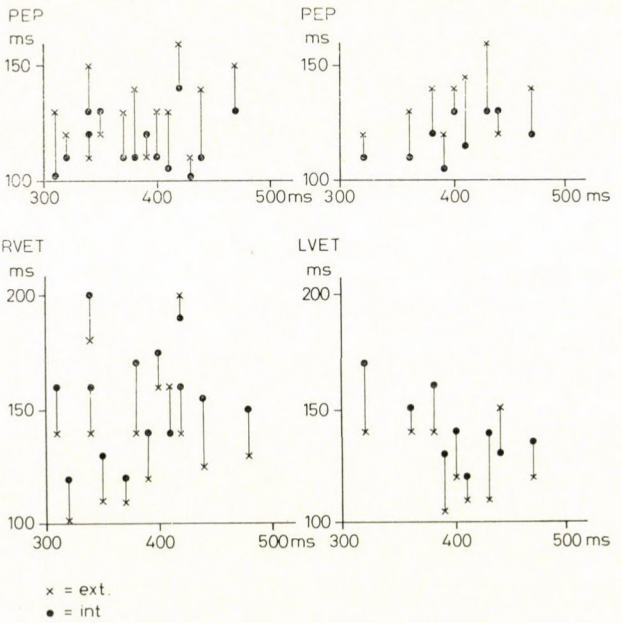

Fig. 3. Comparison of values obtained by indirect (ext.) and by direct (int.) procedures. The abscissa represents the length of the heart cycle, the ordinate the measured data in ms. The lines connecting the respective figures obtained by the direct and by the indirect method mark the magnitude of the differences

found concerning this issue in animals. In our view, there is no real contradiction between the findings. In Table I, we have divided the pre-ejection period into two phases: PEP_1 and PEP_2, which are not identical with PEC_1 and PEC_2 known from he literature. Under PEP_1 we understand the interval between Q and the beginning of the mechanical phenomena, *i.e.* the bottom of the mechanical tracing which corresponds to the electromechanical interval. PEP_2 on the other hand defines the time from the beginning of the bottom of the mechanogram, to its peak, *i.e.* point E which comprizes both the mechanopressor and the isometric phases. It ends with the beginning of ejection marked by point E on the cardiogram. In contrast, PEC_1 denotes the interval from the beginning of the systole to the incisure of the first component of the first heart sound, and PEC_2 the interval from the first component of the first

heart sound to point *E*. Mean duration of the former is 35 msec, that of the latter 67 msec as concerning the left ventricle. The reason why avoided these denotations was because under experimental conditions the notches are difficult to define, while the *Q*-deflection as well as the bottom and the peak of the mechanical tracing can be measured more reliably.

Discussion

As our results show, the pre-ejection period of the left ventricle is longer than that of the right. This is not true for the timings of PEP_1 and PEP_2. As it can be seen from Table I, reversal of contraction occurs in fact earlier in the left than in the right ventricle, the interval between *Q* and the start of the mechanic phenomena (PEP_1) corresponds to 25.6 msec for the left and of 32.5 msec for the right ventricle.

Contraction of the left ventricle thus starts earlier and ends later than that of the right. On the other hand, PEP_1 is shorter, and PEP_2 is longer, for the left than for the right ventricle.

As Table I further shows, the ejection period is very short for both the left and the right ventricle. This may be attributed to the tachycardia of the animals. It seems none the less clear that, in agreement with direct measurements, the ejection period of the right ventricle is longer than that of the left. This finding also lends support to our view that point *E*, *i.e.* the peak of the apex cardiogram, is suitable for the measurement of the beginning of ejection. We found, on the other hand, a wide range of point "O".

Thus, the mechanical tracings derived from the right and from the left ventricle actually reflect the contraction of the respective ventricle. The quantified pre-ejection and ejection periods correspond to data obtained by intracavitary curves. Point *E* has been found to mark the beginning of ejection and thus seems to lend itself to the measurement of the time relationships of the mechanical phases of right ventricular activity.

REFERENCES

1. ANZOLA, J.: Amer. J. Physiol. **185,** 567 (1956).
2. BRAUNWALD, E., SARNOFF, J., STAINSBY, W. N.: Circulat. Res. **6,** 319 (1958).
3. BURCHELL, H. B., VISSCHER, M. B.: Amer. Heart J. **22,** 794 (1941).
4. LANDIS, C., HUNT, W. A., MOE, S. K., VISSCHER, M. B.: Amer. J. Physiol. **129,** 400 (1940).
5. MIHÓCZY, L., VOITH, L. jr.: Orv. Hetil. **114,** 12 (1973).
6. MIHÓCZY, L., VOITH, L. jr.: Bibl. Cardiol. **33,** 18 (1974).
7. RUSHMER, R.: Cardiovascular Dynamics, W. B. Saunders, Philadelphia, London (1970).
8. SOULIÉ (cit. Warembourgh, H., Dubar, P.): Le chronocardiogramme. Expansion Scientifique Francaise, Paris (1967).
9. WIGGERS, C. J.: Amer. J. Physiol. **56,** 415 (1921).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (3—4), pp 187—193 (1974)

GLOMERULONEPHRITIS OF IMMUNOCOMPLEX ORIGIN ASSOCIATED WITH HODGKIN'S DISEASE

By

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Received April 19, 1974

The case of a patient is reported who suffered from Hodgkin's disease and developed nephrotic syndrome. Biopsy revealed membranoproliferative glomerulonephritis of immunocomplex origin. The relation between the nephrotic syndrome and Hodgkin's disease pointed to an aetiological connection.

The association of Hodgkin's disease and nephrotic syndrome is rare. Since Cornic's first report [2] only 32 cases were published in the literature [1—7, 9—11, 13, 15, 19—24, 26—28.]

The light, immunofluorescent and electron microscopic investigations of percutaneous kidney biopsy specimens revealed further details of the structural changes associated with the nephrotic syndrome. In part of the published cases [3, 6, 7], electron microscopy in accordance with the light microscopical picture, showed either a normal structure, or slight glomerular changes. To our best knowledge, only Hardin et al. [7] and Froom et al. [3] have reported cases in which Hodgkin's disease was associated with the nephrotic syndrome, due to membranous glomerulonephritis verified by electron microscopy.

Report of a Case

The patient was a 40 years old male. His disease had begun four years earlier with enlarged lymph nodes on the left side of the neck. Histological examination showed Hodgkin's disease of mixed cellularity. He had had ^{60}Co irradiation of the enlarged lymph nodes and cytostatics. After this treatment the lymph nodes had decreased in size, and the patient had had no complaints. Two years later an exacerbation had occurred, with enlarged lymph nodes on both sides of the neck. Lymph node biopsy had again shown Hodgkin's disease of mixed cellularity. During this exacerbation, transient proteinuria, microscopic haematuria with casts had appeared. The patient was admitted to our Department in January, 1972, in a poor general state of health. He had enlarged lymph nodes on both sides of the neck and moderate oedema of the extremities. The quantity of urine was 50—200 ml daily. The laboratory data pointed to a nephrotic syndrome. Treatment consisted in ^{60}Co irradiation (3×2000 rad) of the enlarged lymph nodes, protein substitution, diuretics, and corticosteroids. Following

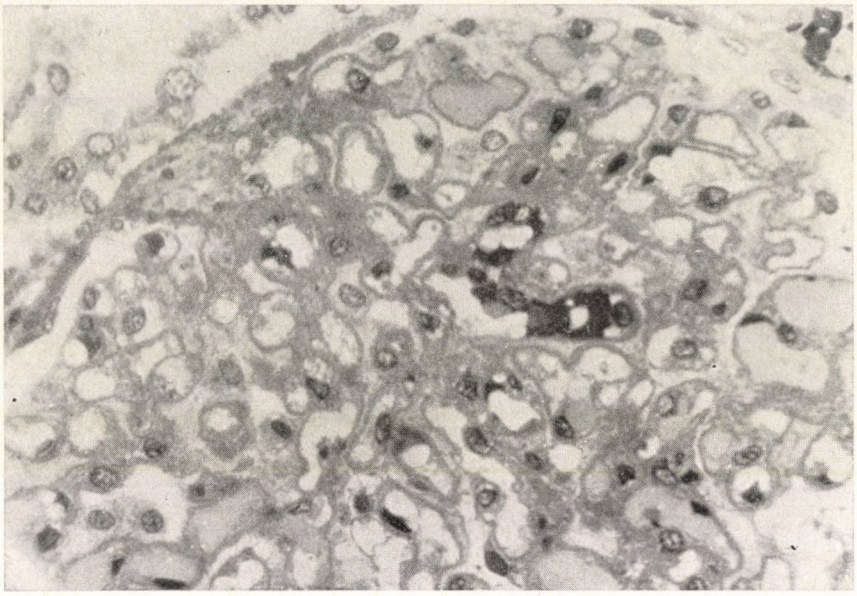

Fig. 1. Light microscopic picture, percutaneous renal biopsy. Increased cellularity, thickened mesangial areas, thickening of basement membrane with narrowing of capillary lumina. Semithin section. Azur III methylene blue. ×600

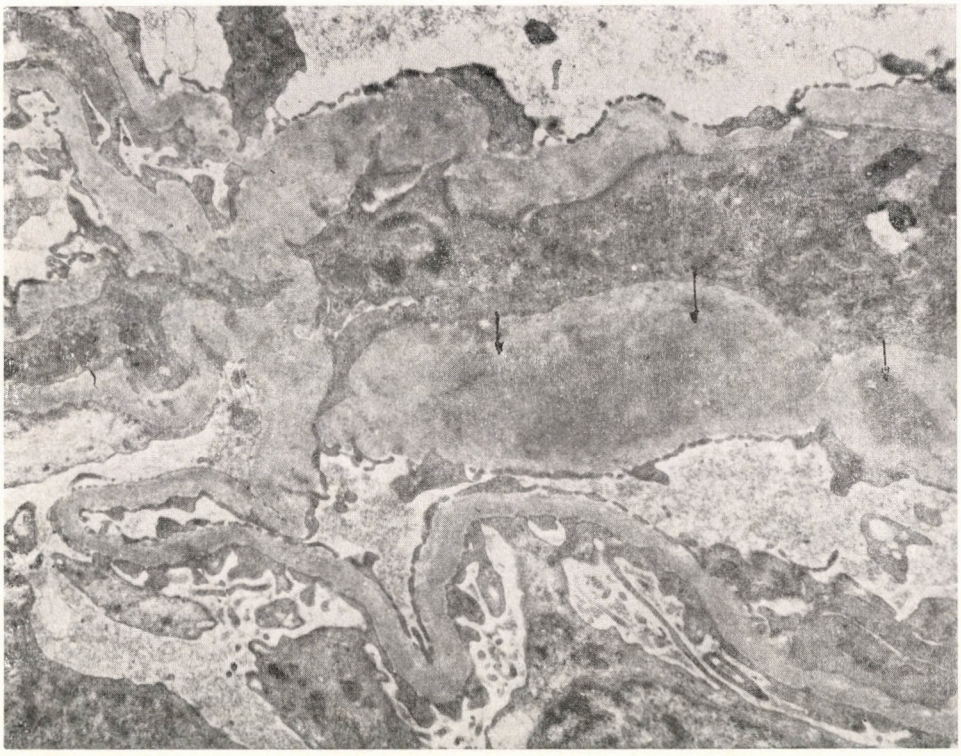

Fig. 2. Electron microscopic picture, showing deposits in basement membrane (arrows). ×22,000

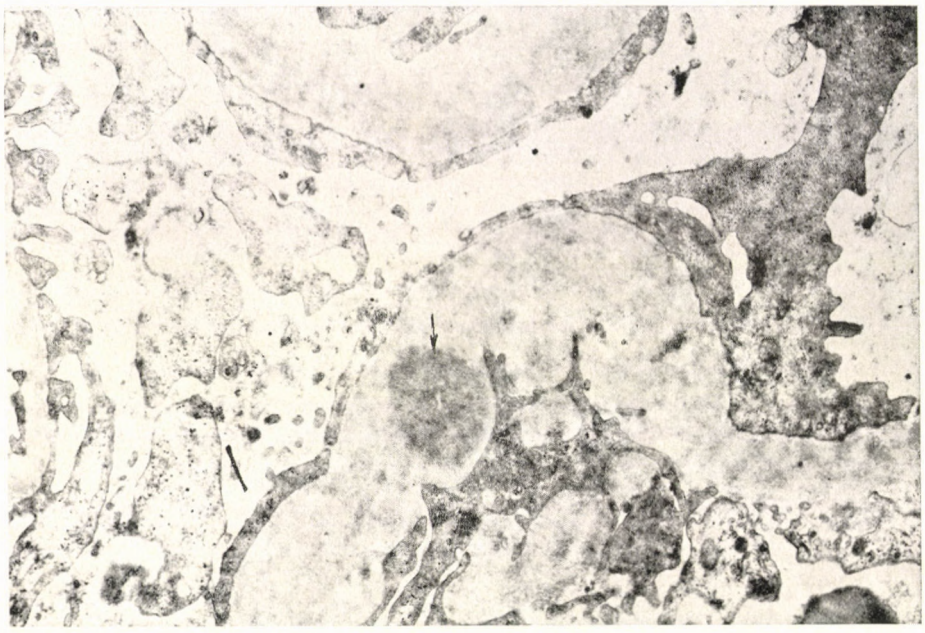

Fig. 3. Electron microscopic picture. Well-circumscribed, intramembranous deposit (arrow). The foot processes had fused. ×22,000

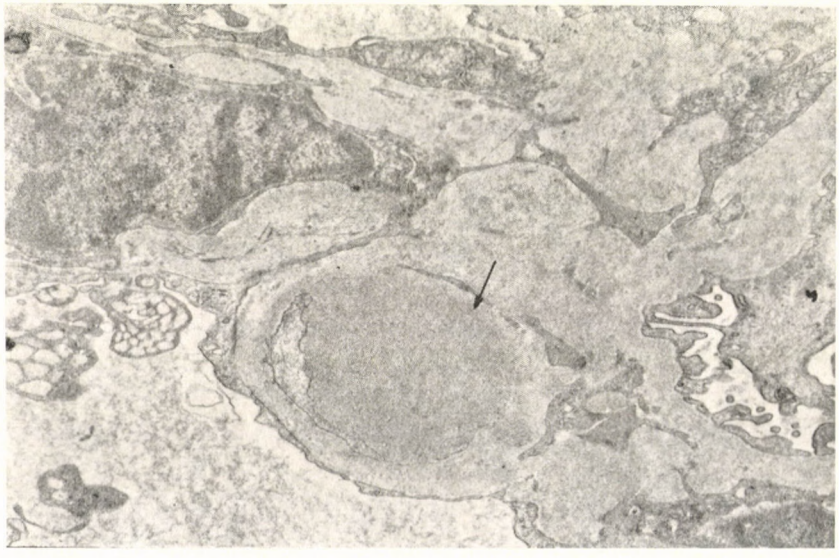

Fig. 4. Electron microscopic picture. Subendothelial deposit (arrow). The foot processes had fused. ×20,000

this treatment, renal function improved and three months later the patient was discharged in a good general condition. One and half months later percutaneous kidney biopsy was carried out, the specimen was investigated by light-, immunofluorescent- and electron microscopy. Light microscopy showed an irregularly thickened glomerular basement membrane and slight mesangial proliferation with some narrowed capillary lumina (Fig. 1). Adhesion between Bowman's capsule and capillaries could be noticed. Electron microscopy revealed irregular thickenings of the glomerular basement membrane by not sharply demarked, nodular intramembranous (Fig. 2), in other areas circum-

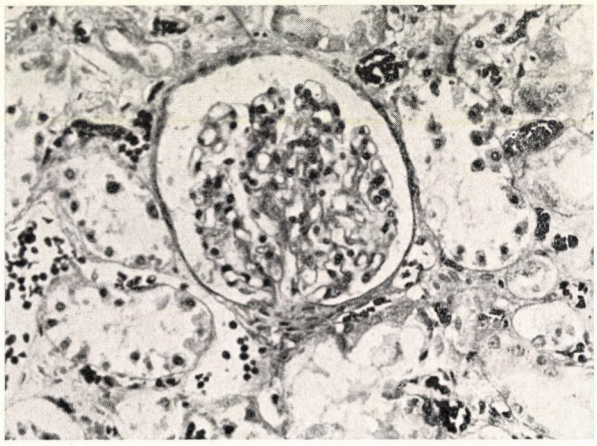

Fig. 5. Light microscopic picture of autoptic kidney. Slightly increased cellularity of glomerulus, without signs of glomerulonephritis. Endes' combined trichrome. ×400

scribed nodular intramembranous (Fig. 3) and subendothelial deposits (Fig. 4). In these areas, the foot processes were fused. The mesangial areas were slightly extended. The whole picture was characteristic of membranoproliferative glomerulonephritis of immunocomplex origin. By fluorescent microscopy, a granular type fluorescence caused by marked anti-IgG effect was detected in the glomerular basement membrane.

Ten months later the patient was readmitted. At this time, no renal disturbances were found, but he died of pneumonia. Necropsy revealed Hodgkin's disease of mixed cellularity in the lymph nodes and the liver. The kidneys were not involved. Autoptic kidneys were investigated by light- and electron microscope. Under the light microscope slightly enhanced cellularity in the glomeruli and in some places thickened mesangial areas were found (Fig. 5), but the picture was not characteristic of glomerulonephritis. Electron microscopy of 15 glomeruli did not detect deposits in the basement membrane, but the glomerular basement membrane was thickened and the foot processes had fused.

Discussion

The association of nephrotic syndrome with Hodgkin's disease may be explained in different ways [23]. In our case the clinical picture and the histological finding suggested the explanation that an antigen-type material from lymphogranulomatous tissue, or the agent(s) causing it, had damaged the basement membrane of the glomeruli directly or by producing immunocomplexes. In agreement with FROOM et al. [3], HANSEN et al. [6] and PLAGER and STUTZMAN [23], we noticed a slight correlation between the activity of the nephrotic syndrome and that of Hodgkin's disease. The disease showed an exacerbation when the nephrotic syndrome had reached the oligo-anuric phase and after ^{60}Co irradiation of the enlarged lymph nodes improvement of Hodgkin's disease was followed by a regression of the nephrotic syndrome. In the patients of BRODOVSKY et al. [1] and TAPIE et al. [28], successful treatment of Hodgkin's disease also resulted in an improvement of the nephrotic syndrome.

Under the effect of cytostatic treatment the improvement could be considered a result of a direct effect on the nephrotic syndrome. Improvement of the nephrotic syndrome following the extirpation or, as in our case, the irradiation of the lymph nodes is in favour of a close causal connection between Hodgkin's disease and the nephrotic syndrome.

In our case, biopsy at the beginning of remission revealed membranoproliferative glomerulonephritis, with deposits pointing to an immunocomplex origin. The immunofluorescent investigation showed granular IgG deposition in the glomerular basement membrane; this too is in favour of the immunocomplex origin of glomerulonephritis. Like in the case of other tumours and sarcoidosis causing nephrotic syndrome [8, 12, 14, 16, 25], in the case of Hodgkin's disease too, the pathogenic agent (virus?) or some material produced by the tumour cells may have induced antibody production, and the immunocomplex could have been bound to the basement membrane of the glomeruli. In our case the association of the two diseases showed a similarity to the NZB mice having lymphomas and their females glomerulonephritis too. MELLORS et al. [17, 18] showed that the causative agent was a murine leukaemia virus, and the antigen was found not only in the lymphomas but also in the glomerular basement membrane. These data point to an aetiological connection between lymphomas and glomerulonephritis. Theoretically, there may be a similarity between the disease of the NZB mice and Hodgkin's disease complicated with glomerulonephritis. VIENNA et al. [29] suppose a barrier held virus to be the causative agent of Hodgkin's disease, by provoking immunocomplex production, and these immunocomplexes would cause the specific lymph node changes. These immunocomplexes may directly damage the glomerular basement membrane, causing glomerulonephritis or

nephrotic syndrome in association with Hodgkin's disease. Direct and unambiguous evidence of this could only be presented by identifying the aetiological factor and the antigen responsible for the immunocomplex process.

Acknowledgements

We are indebted to Miss I. NAGY for technical assistance and to Mr. I. VERSÉNYI for the micrographs.

REFERENCES

1. BRODOVSKY H. S., SAMUEL M. L., MIGLIORE P. J., HOWE C. D.: Chronic lymphocytic leukemia, Hodgkin's disease, and the nephrotic syndrome. Arch. intern. Med. **121,** 71—75 (1968).
2. CORNIC H. J.: Une forme nouvelle de la maladie de Hodgkin. La granulomatose maligne a type de néphrose lipoidique. These 547, Paris (1939).
3. FROOM D. W., FRANKLIN W. A., HANO J. E., POTTER E. V.: Immune deposits in Hodgkin's disease with nephrotic syndrome. Arch. Path. **94,** 547—553 (1972).
4. GHOSH L., MUEHRCKE R. C.: The nephrotic syndrome: A prodrome to lymphoma. Ann. intern. Med. **72,** 379—382 (1970).
5. HAMBURGER J., RICHET C., CROSNIERT J.: Nephrology. Vol. 1. Saunders, Philadelphia (1968).
6. HANSEN H. E., SKOV P. E., ASKJAER S. A., ALBERTSEN K.: Hodgkin's disease associated with the nephrotic syndrome without kidney lesion. Acta med. scand. **191,** 307—313 (1972).
7. HARDIN J. G., COKER A. S., BLANTON J. H.: Medical grand rounds from the University of Alabama Medical Center, Sth. med. J. **62,** 1111—1118 (1969).
8. HAUPT R.: Über Glomerulonephrose bei malignen Tumoren. Zbl. allg. Path. **108,** 80—86 (1965).
9. JACKSON R. H., Oo M.: Nephrotic syndrome with Hodgkin's disease. Lancet **2,** 821 —822 (1971).
10. KIELY J. M., WAGONER R. D., HOLLEY K. E.: Renal complications of lymphoma. Ann. intern. Med. **71,** 1159—1175 (1969).
11. KIY Y.: Sindrome nefrotica associada a doenca de Hodgkin. Rev. Hosp. Clin. Fac. Med. Sao Paulo, **22,** 186 (1967).
12. LEE J. C., YAMAUCHI H., HOPPER J. jr.: The association of cancer with the nephrotic syndrome. Ann. intern. Med. **59,** 265—274 (1963).
13. LESTRADE J.: Néphrose lipoidique et maladie de Hodgkin associées. Thèse 542, Paris (1946).
14. LOUGHRIDGE L., LEWIS M. G.: Nephrotic syndrome in malignant disease of non-renal origin. Lancet **1,** 256—258 (1971).
15. LOWRY W. S., MUNZENRIDER J. E., LYNCH G. A.: Nephrotic syndrome in Hodgkin's disease. Lancet **1,** 1127—1127 (1971).
16. MCCOY R. C., TISHER C. C.: Glomerulonephritis associated with sarcoidosis. Amer. J. Path. **68,** 339—358 (1972).
17. MELLORS R. C., AOKI T., HUEBNER R.: Further implication of murine leukemia-like virus in the disorders of NZB mice. J. exp. Med. **129,** 1045—1062 (1969).
18. MELLORS R. C., HUANG C. Y.: Immunopathology of NZB/B1 mice. V. Viruslike (filtrable) agent separable from lymphoma cells and identifiable by electron microscopy. J. exp. Med. **124,** 1031—1038 (1966).
19. METHA S. R., KUMAR K. K., GUPTA M. L.: Hodgkin's disease with nephrotic syndrome and erythema multiforme. J. Indian med. Ass. **48,** 279—283 (1967).
20. MILLER D. G.: The association of immune disease and malignant lymphoma. Ann. intern. Med. **66,** 507—521 (1967).
21. PASLAWSKA-UDOLF E.: Przypadek nerczycy lipoidowej w przetiegu ziernicy zlosliwej u dziecka 2-letniego. Pediat. Pol. **42,** 591—592 (1967).

22. PERLINSKA-SCHNEJDER L.: Lipoid nephrosis in Hodgkin's disease. An attempt to explain the pathogenesis of the nephrotic syndrome. Pol. Tyg. lek. **20,** 132—137 (1965).
23. PLAGER J., STUTZMANN L.: Acute nephrotic syndrome as a manifestation of active Hodgkin's disease. Amer. J. Med. **50,** 56—66 (1971).
24. RICALENS J.: Contribution a l'étude du syndrome néphrotique au cours de la maladie de Hodgkin—Sternberg. Thèse 94. Toulouse (1956).
25. RICHMOND J., SHERMAN R. S., DIAMOND H. D., CRAVER L. F.: Renal lesions associated with malignant lymphomas. Amer. J. Med. **32,** 184—207 (1962).
26. ROHMER P., SACREZ R.: Un cas de néphrose lipoidique au cours d'une maladie de Hodgkin. Strasbourg med. **108,** 45—47 (1948).
27. SOLSONA CONILLERA J.: Linfogranulomatosis maligna: Forma monoganglionar y nefrósica. Med. clin. (Barcelona) **20,** 37—39 (1953).
28. TAPIE J., LAPORTE J., RICALENS J.: Syndrome néphrotique au cours de la maladie de Hodgkin-Sternberg. Presse Méd. **65,** 287—288 (1957).
29. VIENNA N. J., GREENWALD P., DAVIES J. N. P.: Nature of Hodgkin's disease agent. Lancet **1,** 733—735 (1971).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (3—4), pp 195—199 (1974)

THROMBELASTOGRAPHIC STUDIES AFTER SPLENECTOMY

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Received May 7, 1974

The thrombelastogram was studied in 28 subjects having had splenectomy for various reasons. Signs of hypercoagulability were found all throughout regardless whether splenectomy had been performed for injury in otherwise normal individuals or for hepatic disease. The step phenomenon was not demonstrable in any of the cases.

Although the spleen represents a considerable part of the reticuloendothelial system and of the lymphoid apparatus, its function is not fully understood. The organ is known to serve for the storage of blood, to perform antibody- and lymphocyte-forming functions and to take part in the destruction and removal of erythrocytes, lymphocytes, bacteria, proteins and enzymes [7]. It presumably also takes part in the breakdown of the proteins and enzymatic products of the clotting and fibrinolytic systems, although in this respect its role is not exclusive, since splenectomy is not necessarily followed by any disorder of haemostasis.

Blood coagulation studies including thrombelastography TEG, measurement of the fibrinogen level, clot retraction, prothrombin utilization, and platelet count revealed signs of hypercoagulability after splenectomy for haemolytic anaemia, and even a syndrome consistent with the clinical features of portal vein thrombosis has been described in one case [6]. Other workers found no sign of coagulopathy after splenectomy performed for the same reason [8].

Splenectomy results in clinical cure of certain syndromes associated with haemorrhagic manifestations, such as Werlhof's disease. The question, however, whether clinical cure may be regarded as synonymous with normal blood clotting and fibrinolysis, has yet to be answered.

The aim of the present study has been to ascertain whether a loss of the physiological function of the spleen in normal and in certain pathologic conditions involves any blood clotting abnormality demonstrable by thrombelastography.

Material and method

Thrombelastography (TEG), the method introduced by HARTERT [5], allows to detect abnormalities of clotting and fibrolysis which may be too subtle to be identified by conventional coagulation studies, these reflecting the outcome of the coagulation defect rather than its dynamics.

In the studies, a Hellige type thrombelastograph was used. Blood was withdrawn with a coneless needle, with the apparatus close at hand. The first few ml of blood were discarded, then the blood stream was directed into the cuvette, care being taken to avoid bubbling or turbulence. The cuvette was then placed into the apparatus within 30 sec. Native blood was used because there is some evidence that certain phenomena, in particular the step phenomena, require whole blood for their production [3], although they have been noted after recalcification of citrated plasma too [6]. The parameters determined were r (reaction time), k (clot formation time), mA (maximum amplitude) and E (elasticity), derived from the latter [1, 10]. The thrombelastographic index was also estimated, on the basis of ORLIKOV and SHOPFER's [9] formula,

$$L = \frac{r \, X \, k}{m \, A}$$

Fibrinolytic activity was assessed semiquantitatively and graded + if the amplitude narrowed to 20 mm within 4 hours; ++ if this required 2 hours; +++ if the amplitude fell to 0 within 4 hours or if it attained again 20 mm within 1 hour.

Irregularities or deformations of the TEG, including the step phenomenon were evaluated apart from fibrinolysis, since the two phenomena may be independent of each other [1, 2].

TEG was performed in 28 cases. The patients, 16 males and 12 females, were between 6 and 58 years of age, all having been subjected to splenectomy more than one year earlier. The diagnoses and the values for the TEG-index, are seen in Fig. 1.

Results

Table I shows the mean TEG values in the patients having had splenectomy for injury and for liver disease, and the probability levels obtained by Student's t test against normal controls (Table I). The TEG data of the patients having had splenectomy for the other reasons are presented in Table II.

Fig. 1 shows the TEG-index. In agreement with ORLIKOV and SHOPFER [9] the figures were found to approximate unity in the controls. An increase is indicative of hypocoagulability, a decrease of hypercoagulability (Fig. 1).

In the two groups, the results were as follows. In the group where splenectomy had been made necessary by injury, no significant change in r was noted, in opposition to a significant decrease in the value for k and a significant increase for mA and and E. In the group of liver disease, the value for r was not significantly affected whereas that for k was reduced to the level of significance. This was also true for mA, while the increase in E derived from this parameter was not significant. The significant and non-significant changes consistently pointed to a hypercoagulability in both groups, as illustrated by the diagram where it can be seen that the TEG index was around unity in the controls and below unity in the two patient groups. A TEG index indicative of hypocoagulability was found in 4 cases in the total series of 38 patients, *i.e.*

in one each of the controls, and of the groups of liver disease, Werlhof's disease, and hypersplenism.

A moderate fibrinolytic activity was noted in 6 cases, in 4 of the injury group, and one in the liver disease-group, and in a patient who had been sub-

Table I

	Controls (n = 11)	Injury (n = 10)	Hepatic disease (n = 11)	Probability level (Student)
	12.4			
r		11.2		$0.2 > p > 0.1$
			10.7	$0.1 > p > 0.05$
	8.1			
k		6.4		$p < 0.001$
			6.7	$0.05 > p > 0.02$
	50.3			
mA		56.8		$0.01 > p > 0.001$
			58.3	$0.05 > p > 0.02$
	103.9			
E		132.7		$0.01 > p > 0.001$
			151.6	$0.1 > p > 0.05$

jected to splenectomy for echinococcus. The TEG of a patient with cirrhosis was marked by a bulbiform pattern indicative of enhanced fibrinolysis with hypercoagulability. A step phenomenon was not observed in any of the cases.

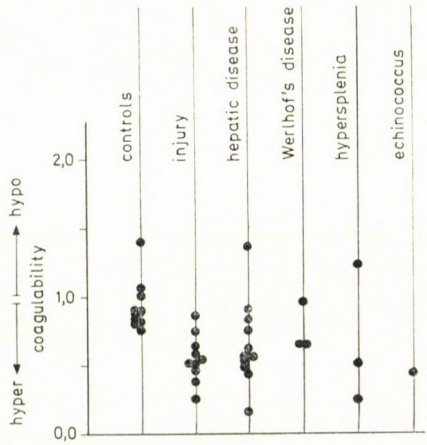

Fig. 1. TEG-index in relation to diagnosis

Table II

No.	Patient	Dg.	t	k	mA
1.	OM.	Werlhof's disease	12.5	8.1	59
2.	SzM.	Werlhof's disease	13.7	7.5	59
3.	UJ.	Werlhof's disease	14.5	10.8	40
4.	GyS.	hypersplenism	10.6	6.2	65
5.	MJ.	hypersplenism	15.0	12.5	37
6.	TA.	hypersplenism	14.5	7.5	50
7.	KM.	echinococcus	16.2	5.6	50

Discussion

It has already been pointed out that while some workers failed to detect any alteration of TEG in subjects having had splenectomy for haemolytic anaemia [8], others noted signs of hypercoagulability [6], including the step phenomenon. An additional deficiency in fibrinolytic activity was common. Changes in the platelet count, and in TEG, including the step phenomenon point to a causal involvement of a deficient number or function of platelets [1]. Similar abnormalities have been noted in leukaemia and in other conditions associated with leukocytosis, the normalization of leukocyte count being accompanied by that of TEG [2, 3, 4]. It has been also suggested that after splenectomy for haemolytic anaemia it is the deficient platelet function which counterbalances the hypercoagulability to an extent that solely the TEG proves its existence, as reflected by the step phenomenon. It was this finding which has prompted us to study the TEG after splenectomy.

The present observations showed that the TEG reveals persistent changes indicative of hypercoagulability, in the presence of normal erythrocyte, leukocyte and platelet counts long years after splenectomy, whether performed for injury in otherwise normal subjects or for liver disease. A step phenomenon has not been encountered in the present cases. The signs of hypercoagulability consisted in a respective increase and a decrease in k and mA, parameters reflecting changes in the number and function of platelets [1, 8, 10], although the role of a potential serum factor affecting the stability of platelets has also been suggested [1].

The present results seem to suggest that the primary cause of the statistically significant, though clinically latent, hypercoagulability after splenectomy lies in abnormalities of platelet function due to the loss of influence exerted by the spleen on the clotting system. Though this concealed hypercoagulability in itself has hardly ever any direct clinical consequence [6], it

involves the hazard of clinical manifestations in the presence of other factors of local or general nature favourable for hypercoagulability, such as varicose veins or the use of oral contraceptives [11]. This implicates that even if splenectomy has been performed for injury in otherwise normal subjects, the existence of a latent hypercoagulability must be taken into account as a factor contraindicating the use of oral contraceptives.

REFERENCES

1. Donner, L.: Probl. Gemat. **4** (fasc. 7) 15—24, 1959.
2. Fukuzawa, M.: Acta Haemat. jap., **25,** 44 (1962).
3. Giacca, S., Negrini, A. C.: Arch. E. Maragliano Pat. Clin. **15,** 1393 (1959).
4. Giacca, S., Negrini, A. C.: Arch. E. Maragliano Pat. Clin. **15,** 1403 (1959).
5. Hartert, H.: Klin. Wschr. **26,** 577 (1948) and Z. ges. exp. Med. **117,** 189 (1951).
6. Karaseva, E. B., H. A. Messineva, I. B. Poloterov: Probl. Gemat. **10** (fasc. 7) 30—34, 1965.
7. Lennert, K., Harms, D.: Die Milz. Springer, Berlin.
8. Nicola, P.: Thrombelastography, Springfield, Ill. 1957.
9. Orlikov, G. A., Shopfer, G. G.: Ter. Arkh. **41** (fasc. 8) 84—87, 1969.
10. Potashov, L. B.: Vesztn Hir. **87** (fasc. 11) 84—87, 1969.
11. Siró, B., Misz, M., Bazsó, J.: Orv. Hetil. **112,** 2822 (1971).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (3—4), pp 201—209 (1974)

QUANTIFICATION OF SPATIAL MAGNITUDE AND VELOCITY OF NORMAL AND ABNORMAL QRS

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Received June 28, 1974

The QRS complex has been studied for spatial magnitude and spatial velocity in 441 clinical cases. Spatial magnitude was derived by means of an analogue computer, the bioelectrically instructed triaxicardimeter. Spatial velocity was computed by automatic analysis. The analogues of spatial magnitude (M), azimuth (H°) and elevation (V°) were converted to digital values after being resolved into instantaneous vectors of 10 m/sec, and the numerical data of the components XYZ were obtained on grounds of the trigonometric functions yielded by the ODRA 1204 computer. Scalar values of spatial velocity were calculated from the values of the components on the basis of the sV-formula. The curves of spatial magnitude and spatial velocity were reproduced from the numerical data on the basis of 10 m/sec units or spatial magnitude, of 1/8 cycles, for spatial velocity, and the averages referred to the individual patient groups (graphic algorhythms) were analyzed.

Spatial magnitude means the vector quantity derived from the coordinates *XYZ*. Quantification of spatial magnitude is based on the Pythagorean spatial theorem:

$$M = \sqrt{Mx^2 + My^2 + Mz^2}$$

where *XYZ* represent the Cartesian coordinates. A polar vector is defined by its magnitude, orientation and the unit of measure corresponding to its physical character. The magnitude of polar vectors is defined by their spatial magnitude, and orientations by the azimuth and elevation angles. Spatial velocity (sV) means the velocity of spread of impulse computed or derived from the coordinates *XYZ*. Quantification of spatial velocity is based on the formula

$$sV = \sqrt{\left(\frac{dx}{dt}\right)^2 + \left(\frac{dy}{dt}\right)^2 + \left(\frac{dz}{dt}\right)^2}$$

where *XYZ* represent the Cartesian coordinates and d*x*, d*y* and d*z* their changes in the function of d*t*. Spatial velocity is a scalar magnitude, definable by one single numerical value and by its unity of measure. Spatial velocity ECG represents the rotation velocity of the spatial VCG loop in a scalar form. Consequently, the farther apart are the individual points of the spatial Lissajous-loop resolved into instantaneous vectors, the greater the rotation velocity of the loop, and conversely.

Definition of both spatial magnitude and spatial velocity require mathematical or automatic analysis by computer techniques. In clinical practice the parameters of spatial magnitude are derived either numerically or graphically. For primary graphical display special-purpose computers, for primary numerical display digital computers are used.

The analogue computer described by SAYERS [1] was suited for the definition of spatial magnitude. The analogue computer of ABILDSKOV et al. [2], MOORE et al. [3] and of ANTALÓCZY et al. [4] is suitable for the definition of spatial magnitude and of its orientation and of the azimuth and elevation angles.

Spatial velocity was defined by HELLERSTEIN and HAMLIN [5]. SANO et al. [6], MORI [7] and KENEDI et al. [8] derived it by means of analogue computation and characterized its normal and abnormal patterns.

YANO and PIPBERGER [9], MACFARLANE et al. [10], RUTTKAY-NEDECKY and RIJLANT [11] have defined the numerical parameters of spatial magnitude and of spatial velocity by automatic analysis and displayed them graphically. In the PIPBERGER-program the data for both spatial magnitude and spatial velocity are computed by automatic analysis from the numerical data of the Cartesian XYZ-coordinate system.

Material and method

Spatial magnitude was determined by means of the triaxicardiometer [12, 13, 14, 15, 16, 17, 18, 19]. Spatial velocity was computed by automatic analysis. The triaxicardiometer (TCM) is an analogue computer fed directly by the biocurrents of FRANK's lead system [20].

The operating units of TCM return the functions

spatial magnitude: $M = \sqrt{Mx^2 + My^2 + Mz^2}$

azimuth: $H^\circ = \text{arc} \cdot \sin \frac{Mx}{Mxz}$

elevation: $V^\circ = \text{arc} \cdot \sin \frac{M}{My}$

The M, H° and V° values provided by the output unit of TCM are registered on a multichannel direct-writing ECG equipment in the form of diagrams, the triaxicardiograms.

The analogues of spatial magnitude (M), azimuth (H°) and elevation (V°) angles were converted to digital values after being resolved into instantaneous vectors of n/10 m/sec units. In the interest of comparability the QRS complexes of varying duration were divided into 8 units. The M, H° and V° values per 1/8 cycle were computed from the respective measured values by linear interpolation. When the width of QRS measures 80 msec, then the values per 1/8 cycle correspond exactly to 10 m/sec. If, however, the width of QRS exceeds 80 msec, as it was the case in the groups with bundle branch block and fascicular block, then the values per 1/8 cycle are obviously above 10 m/sec. The numerical data for the components X, Y and Z were derived from the trigonometric functions yielded by computer ODRA 1204,

$$X = M \cdot \cos \cdot V^\circ \cdot \cos H^\circ$$
$$Z = M \cdot \cos \cdot V^\circ \cdot \sin H^\circ$$
$$Y = M \cdot \sin \cdot V^\circ$$

From the data thus obtained the scalar values of spatial velocity were computed on the basis of the sV formula. The curves of spatial magnitude and spatial velocity were plotted on the basis of the numerical data for spatial magnitude and spatial velocity referred to the 10 m/sec and 1/8 cycles, respectively, and their averages in the individual patient groups (graphic algorythms) were analyzed for configuration.

Results

From the 440 subjects studied, a normal control group and 19 patient groups were formed. Tables I, II and III show the mean values for spatial magnitude and its scatter, and the spatial velocity components of QRS per 10 m/sec and 1/8 cycle.

In Figs 1 and 2, the mean data seen in the Tables are represented graphically. Fig. 1 shows the curves of spatial magnitude, Fig. 2 those of spatial velocity in the patients and controls. The individual tracings refer to the following groups:

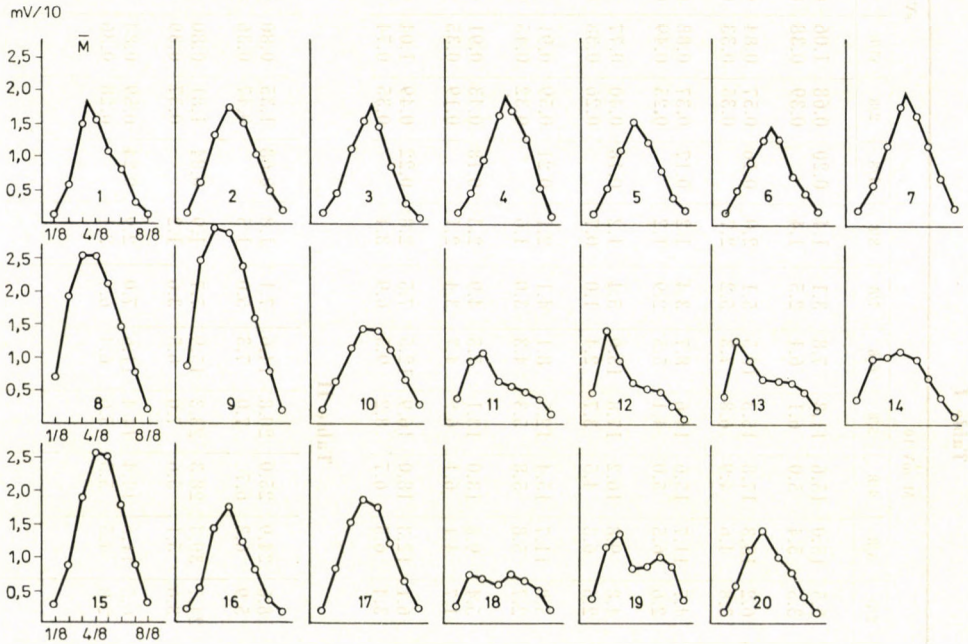

Fig. 1. Graphical algorhythms of spatial magnitude (M) in normal and abnormal cases. The ordinate represents the spatial magnitude in mV/10, the abscissa the corresponding values referred to the 1/8 cycles

1. Normal controls
2. antero-septal infarction
3. extensive anterior infarction
4. antero-lateral infarction
5. postero-diaphragmal infarction
6. extensive posterior infarction
7. antero-posterior infarction
8. left bundle-branch block, uncommon type

Acta Medica Academiae Scientiarum Hungaricae 31, 1974

Table I

Clinical diagnosis	Number of cases	M mV/10								sV m/V/10xmsec							
		1/8	2/8	3/8	4/8	5/8	6/8	7/8	8/8	1/8	2/8	3/8	4/8	5/8	6/8	7/8	8/8
1. Normal controls	40	2.0	6.5	15.0	15.6	11.8	7.8	3.1	1.4	0.20	0.68	1.06	0.98	1.12	0.95	0.59	0.21
		±1.1	3.5	5.4	5.0	6.1	6.1	2.5	1.4		0.39	0.38	0.41	0.47	0.72	0.58	0.16
2. Antero-septal infarction	43	1.6	6.2	13.3	17.8	15.3	10.5	5.1	2.0	0.16	0.57	0.84	0.71	0.74	0.91	0.70	0.39
		±0.9	3.8	4.6	4.9	6.8	7.3	5.2	2.7		0.35	0.33	0.34	0.36	0.37	0.57	0.38
3. Extensive anterior infarction	10	1.7	4.7	11.7	15.6	14.6	8.7	3.4	1.2	0.17	0.37	0.88	0.69	0.84	0.94	0.62	0.28
		±0.8	2.6	6.5	5.0	4.1	5.5	2.9	1.2		0.25	0.49	0.28	0.44	0.46	0.35	0.21
4. Antero-lateral infarction	5	1.8	4.8	9.8	16.2	17.8	13.0	5.4	1.2	0.18	0.40	0.77	1.10	0.80	0.89	0.95	0.49
		±1.0	2.6	6.3	4.5	3.7	2.4	1.0	0.7		0.26	0.38	0.19	0.37	0.47	0.36	0.15
5. Postero-diaphragmal infarction	42	2.1	5.8	11.7	15.4	12.5	8.1	4.1	2.1	0.21	0.59	0.91	1.01	0.95	0.84	0.49	0.24
		±0.9	2.7	5.8	5.8	5.3	4.3	3.0	1.6		0.32	0.45	0.44	0.41	0.46	0.28	0.23
6. Extensive posterior infarction	12	1.8	5.4	9.8	13.0	13.1	7.5	4.9	2.3	0.18	0.43	0.91	0.85	1.21	1.04	0.64	0.34
		±0.7	1.7	4.1	6.1	6.2	4.7	3.4	2.1		0.19	0.35	0.44	0.50	0.50	0.58	0.26
7. Antero-posterior infarction	17	2.2	6.1	12.3	18.0	16.9	12.5	7.5	2.8	0.22	0.49	1.04	0.97	0.90	0.64	0.79	0.61
		±0.9	3.4	6.5	6.7	8.2	9.5	6.9	3.4		0.35	0.74	0.59	0.55	0.29	0.50	0.61

Table II

8. Left bundle-branch block, uncommon type	49	6.8	18.6	24.9	25.0	20.8	14.6	7.1	1.8	0.68	1.35	0.80	0.40	0.62	0.82	0.90	0.67
		±3.3	5.9	6.3	6.7	7.0	7.3	5.0	1.5		0.47	0.36	0.30	0.40	0.35	0.44	0.48
9. Left bundle-branch block, common type	13	8.4	24.7	30.4	28.3	23.3	15.9	7.7	1.9	0.84	1.81	0.80	0.51	0.76	0.99	0.96	0.68
		±1.6	3.6	5.1	5.6	7.0	6.5	3.6	1.2		0.37	0.39	0.37	0.31	0.32	0.37	0.37
10. Left anterior hemiblock	43	2.4	6.5	11.6	14.4	14.4	11.6	7.0	2.9	0.24	0.59	0.84	1.02	0.95	0.76	0.73	0·50
		±0.9	2.8	4.5	5.5	5.6	6.4	6.4	2.1		0.28	0.36	0.59	0.48	0.46	0.59	0.53

11. Right bundle-branch block, classic form	7	4.0	9.5	10.8	6.8	5.8	5.1	3.8	1.7	0.40	0.74	0.59	0.96	0.47	0.18	0.19	0.22
		±1.3	4.5	6.9	2.8	2.9	2.3	1.8	1.0		0.51	0.22	0.49	0.36	0.11	0.14	0.09
12. Right bundle-branch block, uncommon type	9	4.8	14.2	9.7	6.5	5.6	4.9	3.2	1.2	0.48	1.13	1.09	0.93	0.45	0.22	0.22	0.22
		±1.6	4.5	4.2	2.3	1.3	1.6	1.8	1.2		0.40	0.33	0.34	0.27	0.10	0.10	0.11
13. Right bundle-branch block, common type	26	4.3	12.8	9.7	6.8	6.6	6.6	5.1	2.4	0.43	1.07	1.03	1.00	0.49	0.32	0.21	0.31
		±2.1	4.5	5.0	3.5	2.7	2.7	2.9	1.6		0.37	0.58	0.55	0.26	018	0.13	0.18
14. Left anterior hemiblock with right bundle-branch block (Lenègre's syndrome)	11	4.0	10.0	10.2	10.8	10.1	7.3	4.1	1.5	0.40	0.85	0.95	1.10	0.60	0.53	0.38	0.28
		±1.7	4.2	3.1	5.1	5.5	3.9	2.5	1.0		0.34	0.52	0.29	0.22	0.23	0.25	0.19

Table III

15. Left ventricular hypertrophy	58	2.9	8.7	19.3	25.8	25.1	18.1	8.6	2.9	0.29	0.81	1.31	1.28	1.24	1.28	1.24	0.72
		±1.3	4.8	7.5	7.4	9.0	9.9	7.1	2.5		0.42	0.51	0.41	0.61	0.63	0.77	0.64
16. Tight ventricular hypertrophy	12	2.2	5.4	14.7	17.6	12.0	8.0	3.8	1.5	0.22	0.51	1.18	0.71	1.17	1.11	0.60	28.1
		±0.8	3.8	8.6	4.8	4.3	5.7	1.4	0.8		0.45	0.64	0.30	0.48	0.58	0.55	0.0
17. Left anterior hemiblock and antero-septal infarction	18	2.3	8.5	15.4	18.9	17.2	12.2	5.6	2.4	0.23	0.73	0.81	0.77	0.66	0.69	0.84	0.55
		±1.4	5.0	6.1	6.3	6.5	6.7	4.	2.2		0.43	0.29	0.38	0.32	0.25	0.61	0.39
18. Right bundle branch-block and antero-septal infarction	4	2.8	7.6	7.0	6.5	7.4	6.8	5.3	2.3	0.28	0.57	0.46	0.85	0.49	0.21	0.19	0.49
		±1.0	3.2	2.3	4.2	2.2	1.8	1.7	1.1		0.22	0.17	0.18	0.31	0.13	0.06	0.37
19. Lenègre's syndrome and antero-septal infarction	5	3.7	11.6	13.7	8.2	8.6	10.0	8.0	3.4	0.37	0.85	0.73	1.24	0.82	0.42	0.25	0.47
		±1.0	2.9	5.0	5.3	2.7	2.6	2.6	1.2		0.26	0.22	0.46	0.25	0.15	0.08	0.17
20. Left anterior hemiblock and postero-diaphragmal infarction	17	1.9	5.5	11.4	14.2	10.1	7.8	3.9	1.7	0.19	0.50	0.88	1.03	1.06	0.74	0.52	0.30
		±1.0	3.3	5.1	5.8	4.9	4.5	1.4	0.8		0.28	0.48	0.56	0.52	0.38	0.37	0.19

9. left bundle-branch block, common type
10. left anterior hemiblock
11. right bundle-branch block, classic form
12. right bundle-branch block, uncommon type
13. right bundle-branch block, common type
14. left anterior hemiblock with right bundle-branch block (Lenègre's syndrome)

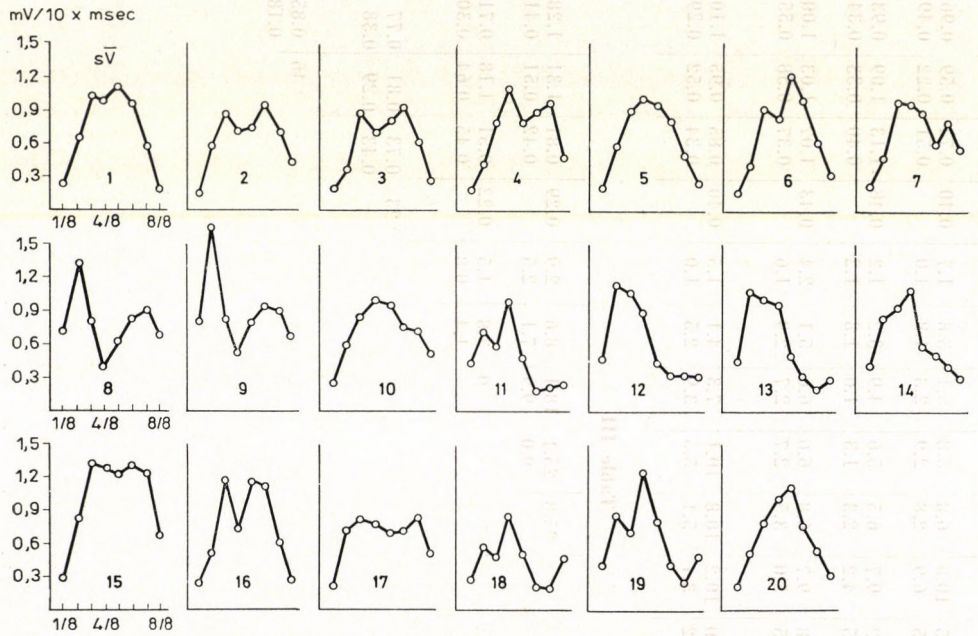

Fig. 2. Graphical algorhythms of spatial velocity in normal and abnormal cases. The ordinate represents the spatial velocity in 0.1 mV/sec, the abscissa the corresponding values referred to the 1/8 cycles

15. left ventricular hypertrophy
16. right ventricular hypertrophy
17. left anterior hemiblock and antero-septal infarction
18. right bundle branch-block and antero-septal infarction
19. Lenegre's syndrome and antero-septal infarction
20. left anterior hemiblock and postero-diaphragmal infarction

Discussion

Maximum mean spatial magnitude corresponds under normal conditions on the evidence of our data and according to PIPBERGER, to 16 mV/10. In postero-diaphragmal infarction and in extensive infarctions the value is smaller,

while in infarctions of other types it usually varies within the normal range. YANO and PIPBERGER [9] found subnormal values in all infarction groups. In these there is a delay in the peak of M: it measures 42 to 46 m/sec against 35 m/sec in normal cases. The intrinsic deflection of the spatial magnitude thus widens. The ascending limb of M often exhibits an initial break which corresponds to a widened Q in the conventional ECG. In left bundle-branch block, the spatial magnitude is of a high amplitude with no break. In reality, the M is wide but for the sake of comparability we have found it convenient to divide the QRS-complexes of variable duration into eight equal parts and to express the values per 1/8 cycle. In left ventricular hypertrophy the spatial magnitude curves are high and may be slightly widened in accordance with the successive stages of hypertrophy. The J-junction is, however, less sharply demarcated from the ST-segment than in left bundle-branch block, furthermore the elevation of ST and the tallness of T are also less marked. In right bundle-branch block, M is low and its values successively decrease after the 4/8 point of QRS. YANO and PIPBERGER [9] reported on similar findings. In Lenègre's syndrome there is a plateau corresponding to the peak of M. In reality, in right bundle-branch block the triaxicardiogram is marked by a deep, distinct dip separating the electric activity of the right and of the left ventricle. In the M-algorythms of the cases of right bundle-branch block presented here the dip is less marked, owing to superposition of the individual components as a result of the division of QRS into eight equal parts. However, the spatial magnitudes in right and left bundle branch block are typical enough to be conclusive of these processes.

Under normal conditions, the spatial velocity ECG exhibits two peaks denoted by SANO et al. [6] ϱ_1 and ϱ_2; the initial peak being smaller than the terminal one. This is in accordance with the present findings [6, 8, 10]. In the present groups of posterodiaphragmal infarction, left anterior hemiblock and right bundle-branch block of uncommon type, the sV ECG showed but one peak. In myocardial infarction of postero-diaphragmal site, YANO and PIPBERGER [9] too have found a single peak only. On the other hand, in the groups of antero-lateral infarction, anterio-posterior infarction and left bundle-branch block, the initial peak of the sV ECG is taller than normal. According to MACFARLANE et al. [10] and SANO et al. [6] too, sV is increased in left ventricular hypertrophy but decreases, as compared with normal values, in all infarction groups.

In left ventricular hypertrophy, the distance between the two peaks is wide. The initial and the terminal peaks are approximately equal in extensive anterior infarction, in antero-septal infarction associated with left anterior hemiblock, and in right and left ventricular hypertrophy. While in antero-septal infarction, YANO and PIPBERGER [9] found two practically equal peaks, according to SANO et al. [6], in interior infarction the first peak is higher than the second. On the other hand, the second peak is much higher than the first in

extensive posterior infarction, in right bundle-branch block, in Lenègre's syndrome, in right bundle-branch block associated with anteri-spetal infarction and in Lenègre's syndrome associated with antero-septal infarction. The value for the initial sV-peak is increased in left bundle-branch block and in left ventricular hypertrophy, while that for the terminal sV-peak is decreased in right bundle-branch block and in Lenègre's syndrome. KENEDI et al. [8] also found a decrease in amplitude of the terminal portion of sV.

As proposed by MACFARLANE et al. [10], sV can be represented in the coordinates of sV and M too, by plotting the maximum values for sV and M. Though the data thus obtained are to some extent more informative, the data relative to sV have in our view little to add to the informative value of the spatial magnitude. This is the opinion of MACFARLANE et al. [10] too. The sV ECG in itself is of little informative value. According to YANO and PIPBERGER [9] "spatial velocity did not contribute more than 1.5% in diagnostic recognition rates". On the other hand, spatial magnitude even in itself provides valuable electric information in various respects. For instance, in right and left bundle-branch block and in hypertrophy of either ventricle, the spatial magnitude is so characteristic as to offer essential diagnostic indications at the first glance. The polar vectors (spatial magnitude, azimuth and elevation), on their part, contain all informations provided by the heart as a bioelectric generator. All electropathological syndromes are definable and quantifiable on the basis of the polar vectors.

REFERENCES

1. SAYERS, B., MCA.: Amer. Heart J. **49,** 336 (1955).
2. ABILDSKOV, J. A., HISLEY, B. L. INGERSON, W. E.: Amer. Heart J. **55,** 104 (1958).
3. MOORE, A. D., HARDING, P., DOWER, G. A.: Amer. Heart J. **64,** 382 (1962).
4. ANTALÓCZY, Z., SOLTI, E., HORVÁTH, K.: A procedure for direct determination of the magnitude and orientation of the cardiac vectors, and an equipment suited for effectuation of the procedure. Hungarian patent 157 366(A 61 b) February 7th, 1968.
5. HELLERSTEIN, H. K., HAMLIN, R.: Amer. J. Cardiol. **6,** 1049 (1960).
6. SANO, T., SUZUKI, F., TAKAHASKI, T., FUKAMACHI, M., FURUKAWA, T.: Jap. Heart J. **8,** 301 (1967).
7. MORI, H.: Jap. Circulat. J. **35,** 791 (1971).
8. KENEDI, P., MÜLLER, GY., SZÉKELY, A.: Cardiol. hung. **1,** 48 (1972).
9. YANO, K., PIPBERGER, H. V.: Circulation **29,** 107 (1964).
10. MACFARLANE, P. W., MITCHELL, J., LAWRIE, T. D. V.: Spatial velocity of the heart vector. XII. International Colloquium Vectorcardiographicum, Brussels 1971. Presses Académiques Européens. Brussels 1972.
11. RUTTKAY-NEDECKY, I., RIJLANT, P.: The angular and spatial velocities of the QRS loop in its own reference plane. XII International Colloquium Vectorcardiographicum, Brussels 1971. Presses Académiques Européennes Brussels 1972.
12. ANTALÓCZY, Z.: A szív elektromos működésének vizsgálata. Medicina, Budapest 1972.
13. ANTALÓCZY, Z , SOLTI, E., HORVÁTH, K.: Orvos és Technika 1971. 3.
14. ANTALÓCZY, Z.: Z. Kreisl. Forsch. **60,** 501 (1971).
15. ANTALÓCZY, Z.: Acta Med. Acad. Sci. Hung. **28,** 119 (1971).
16. ANTALÓCZY, Z.: A new type analogue computer (triaxicardiometer) for the determination of spatial vector. XII International Colloquium Vectorcardiographicum, Brussels 1971. Presses Académiques Européennes, Brussels 1972.

17. ANTALÓCZY, Z., STROMMER, M., REGŐS, L.: Magy. Belorv. Arch. **25,** 314 (1972).
18. ANTALÓCZY, Z., STROMMER, M., REGŐS, L., TOMOR, B.: Automatic vector analysis of myocardial infarction by the triaxicardiometer analogue computer. Journées d'Informatique Medicale, Tome 2. Colloques IRIA, Toulouse 1973.
19. ANTALÓCZY, Z., STROMMER, M., REGŐS, L., TOMOR, B.: Quantitative vector analysis of bundle branch blocks by the triaxicardiometer analogue computer. II. International Symposium on Electrocardiology (XIV. Colloquium vectorcardiographicum): New trends in Electrocardiology. Yerevan 1973.
20. FRANK, E.: Circulation **13,** 737 (1956).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (3—4), pp 211—218 (1974)

IMMUNOLOGICAL ASPECTS OF CHRONIC PYELONEPHRITIS. CELLULAR IMMUNE RESPONSE IN CHRONIC PYELONEPHRITIS

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Received July 22, 1974

The rosette test was used for the study of immunological sensitization associated with chronic pyelonephritis, the number of rosette forming cells being, in the presence of specific antigens, an indicator of the degree of immune response elicited by the antigen. The rosette test proved positive in most acute recurrences of chronic *E. coli* pyelonephritis. This was not the case in Pseudomonas and Proteus infections as far as it could be judged from the O-antigen of these pathogens. The immune response was poor in infections of the lower urinary tract. These findings suggest that immune responses of any major degree are confined to infections affecting the renal parenchyma and even here they are transitory and insufficient for protection from recurrence and reinfection. Therapeutic attempts must therefore be directed against persistence of the pathogen or of their antigens, at the site of the immune reaction, *i.e.* in the interstitial tissue of the kidney.

If an infective process takes a chronic course, then the immune mechanisms are probably at fault. Their failure merits extensive studies as they provide a possibility for reinfections.

The kidney and the urinary pathways are engaged in the removal of waste products. These products move in a centrifugal direction and under normal conditions the surfaces of the urinary apparatus communicating with the outside world allow no access to antigenic substances. This is the reason why in the course of phylogeny and ontogeny the excretory organs have failed in developing protective immune structures comparable in efficiency to those of the respiratory organs (mediastinal lymph nodes) or of the digestive tract (Peyer's patches). The prevalence of chronic urinary infections justly raises the question whether the immune system is capable of adequate immune responses opposing the antigenic, in this case bacterial invasion. In respect of chronic pyelonephritis (CPN), research has largely been concerned with the identification of circulating antibodies to bacterial antigens. It has been found that in acute pyelonephritis the titres of antibody to the O-antigen of the infective agent (*E. coli*) are often increased [1, 2, 3, 4], and decline subsequently with the progress of the process to the chronic stage. In later course, the levels of this antibody are variable and no longer distinctive [5]. When pyelonephritis has reached the chronic stage, any rise in the level of *E. coli* antibody is

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confined to the periods of acute recurrence [6, 7]. Moreover, high titres provide no safeguard against recurrences or reinfections. So the measurement of the circulating antibodies has no any significant diagnostic or therapeutic value.

These considerations have prompted us to study the development of pyelonephritis into chronic form at the level of cellular immunocompetence. In the experiments to be reported we have tested in CPN the patients' T and B lymphocytes for their responsiveness to the antigen of the isolated infective agent and to the antigen of randomly selected strains.

Material and method

Patients, bacterial strains. 18 patients with CPN and 10 with infections of the lower urinary tract were studied. To be included in the study, their urinary cultures had to yield repeatedly the same strains of the same antibiotic sensitivity. *E. coli* was isolated from 14, *Ps. pyocyanea* from 2, *Proteus vulgaris* from 2 CPN-patients. The cultures of all patients with infections of the lower urinary tract yielded E. coli. Serotyping of the strains was not done. As a control, either a randomly selected *E. coli* strain was used, or the *E. coli* U5-41 strain of known antigenic structure (1 : 1L : 7).

Preparation of O antigen. The isolated pathogens were cultured on slant agar for 24 hours from which a suspension in 5 ml physiological saline was prepared and boiled at 100 °C for 1 hour. Then the suspension was made up with physiological saline to a final protein concentration of approximately 2 mg/ml, and treated in an ultrasonic disintegrator type MSE (1.5 A, 1 min) so as to make the O antigen more suitable for the tests.

Antigen adsorption. The O antigen thus prepared was adsorbed onto defibrinated, three times washed and tannin-treated human erythrocytes, in the original way described by BOYDEN [8].

Immunoadherence tests. The procedure described by BIOZZI et al .[9] was used with some modifications. Heparinized blood was left to be sedimentated with 3% gelatin for 45 min, then the supernatant was collected, centrifuged, and the lymphoid cells were separated on Ficoll gradient to 95% purity [10].

Having adjusted the lymphocyte count to 25,000/μl, 0.2 ml of the antigen-coated erythrocytes was added to 0.2 ml of the lymphocyte suspension to give a lymphocyte/erythrocyte proportion of approximately 1 : 100. The mixture was centrifuged at 300 *g* 10 min and incubated for another 15 min. The cell deposit was resuspended in nutrient medium and the number of rosette forming cells (RFC) per thousand lymphocytes was counted under the microscope. Counts over 3 : 1000 were regarded as positive.

Results

The RFC counts of the 18 CPN-patients are shown in Fig. 1. *E. coli* infection was associated with high RFC-figures in 12 of 14 cases. This was not the case in infections with Pseudomonas and Proteus. In approximately 50% of the cases, positive RFC-counts, although of a slighter degree, were obtained with the control antigen (Fig. 1).

In some cases the RFC-counts were checked in successive stages of the disease. Fig. 2 illustrates a case in which the first test yielded a high RFC-count. Three months after chemotherapeutic elimination of the responsible *E. coli* strain, superinfection ensued and the tests were repeated with both strains. While sensitivity to the original strain gradually ceased, that to the reinfecting

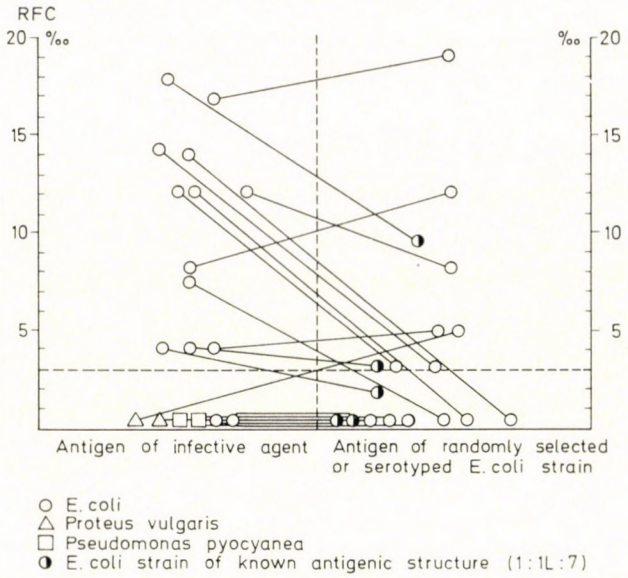

Fig. 1. Number of rosette forming cells (RFC, 1 per 1000) in patients with chronic pyelonephritis

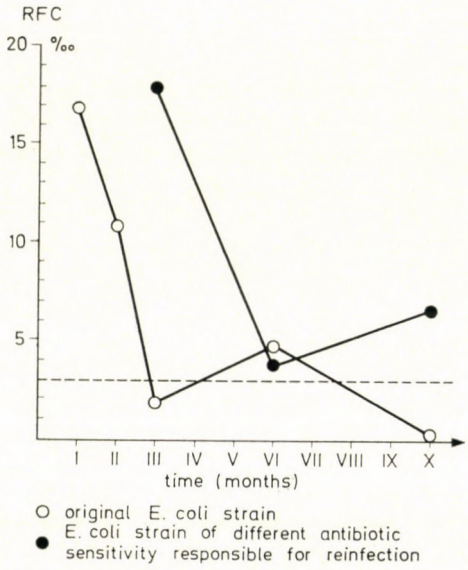

Fig. 2. RFC-counts associated with infection and subsequent reinfection in a case of CPN

strains was still demonstrable at the time of the last test. This was consistent with the clinical course, the new strain having proved drug-resistant.

In contrast, in *E. coli* infections of the lower urinary tract (cystitis, urethrocystitis) only a moderate elevation of the RFC-count was found (Fig. 3),

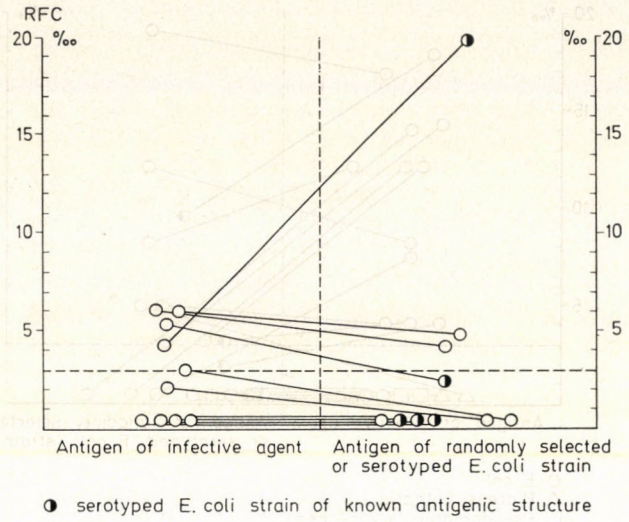

Fig. 3. RFC-counts in infections of the lower urinary tract

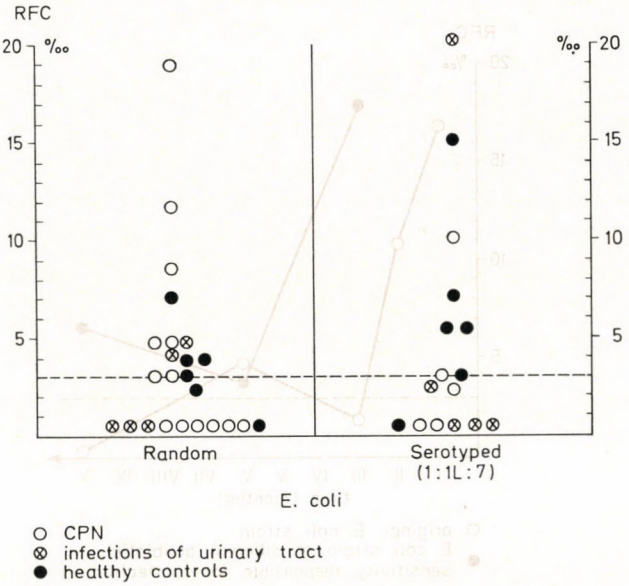

Fig. 4. RFC-responses to the O-antigens of randomly selected and of serotyped *E. coli* strains in infections of the uropoietic apparatus

and even this in approximately 50% only. Simultaneous control examinations with random or serotyped *E. coli* antigen were mostly negative.

Finally, the response in 6 normal subjects, in 10 subjects with infection

of the lower urinary tract and in 18 subjects with CPN to randomly selected and to standard *E. coli* strains was examined. Positive RFC-counts were found to occur in all three groups, thus also in healthy subjects. (Fig. 4)

Discussion

In an earlier study discussing the immunology of CPN it has been pointed out that the titres of circulating antibodies are of little diagnostic value [11]. Studies concerned with the fate of the antigen of the infective agent at the site of infection (bladder, renal pelvis, kidney), in connection with the local immune response to these antigens, have been more rewarding. SANFORD et al. [12] and COTRAN [13] succeeded in localizing the *E. coli* antigen in experimental models, while AOKI et al. [14] and SCHWARTZ and COTRAN [15] in human kidneys, long months after infection. Moreover, IgG production was found to increase significantly in the wall of the infected bladder [16] and in the kidney [17]. 0.5 to 17% of IgG identifiable in the pyelonephritic kidney was found to give a specific reaction with the antigens of the infective agent [18]. On the other hand, the procedure by which these results had been obtained may have not provide any indication whether these specifically reacting immunoglobulins were actually circulating antibodies carried with the blood stream to the infection site or local products of immunocytes having gained access to the kidney, or whether they had resulted from the locally destroyed immunocytes. MILLER and NORTH [19] demonstrated chiefly antibodyforming B-immunocytes in the kidney with the plaque-method and found that the immunoglobulins formed at the infection site were prevalently of the IgM type, thus not identical with the circulating antibodies which had been identified as IgG. Moreover, no relationship was demonstrable between the number of local immunocompetent cells and the titres of circulating antibodies. The specific antibody found in the urine proved to be of renal origin.

The data presented above have been derived mainly from animal experiments and primarily concern immunoglobulins formed in response to the infective agent in the kidneys and urinary passages.

The aim of the present study has been to assess the immune responsiveness of lymphoid cells by means of the rosette test in human CPN, in the acute stage of the process marked by bacteriuria. In 12 of 14 cases we found a significant increase in the number of immunocytes generated by the antigenic stimulus of the circulating infective bacterial agent. By following up the process we have been able to ascertain that when reinfection ensues after eradication of the earlier infective agent, then the original antigenic stimulus gradually

fades, while the immune response is directed against the new antigen reacting on the antigen change of the reinfection.

Though the present observations are not extensive they clearly show that the immune system responds poorly to the O-antigens of *Pseudomonas pyocyanea* and *Proteus vulgaris*. This may be the reason why these bacterial strains are of higher virulence than *E. coli* in urinary as well as in other infections. It is, however, possible that in the case of *Ps. pyocyanea* and *Pr. vulgaris*, the antigens K and H provide the essential antigenic stimuli for the immune responsiveness of the host. (Owing to technical obstacles we have not been able to test the antigens K and H.)

It is furthermore clearly illustrated by the present observations that infections of the upper urinary passages provide a more powerful antigenic stimulus than do those of the lower urinary tract, as reflected by the respective RFC counts.

The rosette test was consistently positive with the control *E. coli* strain in 20 to 30% of the cases. This is hardly surprising in view of the close similarity in antigenic structure of the enterobacteria [20]. The O-antigen of *E. coli* has been detected by immunofluorescence in surgically removed pyelonephritic kidneys by means of immune serum to O-antigen of *E. coli* of a different strain in 30 to 90% of the caes [14, 15], thus providing confirmatory evidence of the common antigenic origin. Recently, KUDO [21] has pointed to the presence of a common antigen in the membranes of Gram-negative bacteria, and found the titres of antibody to this membrane antigen below 1 : 8 in 98% of healthy males and in 90% of healthy females. On the other hand, non-urinary infections (dysentery, infections of the biliary tract) were associated in 20 to 40%, infections of the lower urinary tract in 60 to 80%, acute pyelonephritis in 40 to 60%, chronic pyelonephritis in 90% with abnormally high titres of antibody to the common antigen. It was also possible to identify the bacteria or their antigenic breakdown products in the kidney by means of immunofluorescence, making use of labelled antiserum to the common antigen. In the present study, nonspecific positivity against the common antigen was of much the same frequency whether randomly selected or invariably identical *E. coli* strains had been used as control (Fig. 4).

The *in vivo* phenomena of cellular immunity are but incompletely reflected by rosette formation *in vitro* and by the RFC count. Yet, the present results seem to justify certain conclusions concerning the "immunity" to *E. coli* in CPN. These may be summed up as follows.

1. Infections of the lower urinary tract fail to elicit any significant immune response on the part of the immunocompetent cells, elevation of antibody titres being also uncommon in these conditions. In this manner, spread of the infection meets no obstacle of immunological nature and the infection may become chronic.

2. Though there is some immune response in CPN, it is weak and vanishes completely within 2 to 3 months after the cure or control of the infection, thus affording no protection against reinfection.

3. The fact that the immune response is feeble for affording adequate protection, confines the therapeutic attempts to the prevention of bacterial invasion and of the persistence of bacterial antigens in the kidney. To employ immunosuppressive drugs for this purpose would be feasible only if their effect could be limited to the suppression of the intrarenal immunological processes so that they do not destroy the organ. This is, however, still beyond our possibilities.

REFERENCES

1. WINBERG, J., ANDERSEN, H. J., HANSON, L. A., LINCOLN, K. R.: Studies of urinary tract infections in infancy and childhood. I. Antibody response in different types of urinary tract infections by coliform bacteria. Brit. med. J. **2,** 524 (1963).
2. ANDERSEN, H. J., BERGSTRÖM, T., LINCOLN, K., OERSKOV, F., WINBERG, J.: Studies of urinary tract infections in infancy and childhood. VI. Determination of *E. coli* antibody titers in the diagnosis of acute urinary tract infections lacking the usual urinary findings. J. Pediat. **67,** 1080 (1965).
3. PERCIWAL, A., BRUMFITT, H. W., LEUVOIS, J. D.: Serum-antibody levels as an indication of clinically disapparent pyelonephritis. Lancet **2,** 1027 (1964).
4. BRUMFITT, W.: Urinary tract infections. Localization of the site in the infection and its effects upon treatment. Proc. roy. Soc. Med. **58,** 783 (1965).
5. SZABÓ, T., FODOR, M., BOBORY, J., PETRÁNYI, GY.: A chronicus pyelonephritis immunológiai vizsgálata. Rheum. Baln. Allerg. **10,** 57 (1969).
6. KAARSALO, E., KASANEN, A., LAURENT, B., PIIRONEN, O., RAUNIO, V.: Occurrence of *E. coli* agglutinins in the sera of patients with coli pyelonephritis. Ann. Med. Intern. Fenn. **51,** 31 (1962).
7. KLAREA, B., KOCH, H., SCHMIDT, H.: Coli Antikörpertiter bei Harnweginfektionen. Dtsch. Gesundh.-Wes. **27,** 827 (1972).
8. BOYDEN, S. V.: The adsorption of proteins on erythrocytes treated with tannic acid and subsequent agglutination by antiprotein sera. J. exp. Med. **93,** 107 (1951).
9. BIOZZI, G., STIFFEL, C., MONTON, D., LIACOPOULOS-BRIOT, M., DECEREUSEFOUD, C., BOUTHILLER, Y.: Étude de phénomene de l'immunocyto-adherence au cours de l'immunisation. Ann. Inst. Pasteur **110,** 7 (1966).
10. DOUGLAS, R., FIGGINS, K. P.: A rapid micro-method for the preparation of lymphocyte suspensions. N. Z. J. Med. Lab. Technol. **25,** 17 (1971).
11. PETRÁNYI, GY.: A chronikus pyelonephritis immunológiája. Orv. Hetil. **109,** 841 (1968).
12. SANFORD, J. P., HUNTER, B. W., DONALDSON, P.: Localization and fate of *Echerichia coli* in haematogenous pyelonephritis. J. exp. Med. **116,** 285 (1962).
13. COTRAN, R. S.: Retrograde proteus pyelonephritis in rats: localization of antigen and antibody in treated sterile pyelonephritic kidney. J. exp. Med. **117,** 813 (1963).
14. AOKI, S., IMAMURA, S., AOKI, M., MCCABE: Abacterial and bacterial pyelonephritis: detection of bacterial antigen. Clin. Res. **17,** 470 (1969).
15. SCHWARTZ, M. M., COTRAN, R. S.: Common enterobacterial antigen in human chronic pyelonephritis and interstitial nephritis. An immunofluorescent study. N. Engl. J. Med. **289,** 830 (1973).
16. HAUD, W. L., SMITH, J. W., SANFORD, J. P.: The antibacterial effect of normal and infected urinary bladder. J. Lab. clin. Med. **77,** 605 (1971).
17. SPENCER, A. G., FAIRHEAD, R.: The cellular immune response in experimental *E. coli* pyelonephritis in the rat. Nephron **9,** 325 (1972).
18. LEHMANN, J. D., SMITH, J. W., MILLER, T. E., BARNETT, J. A., SANFORD, J. P.: Local immune response in experimental pyelonephritis. J. clin. Invest. **47,** 2541 (1968).

19. MILLER, T. E., NORTH, D.: Studies of the local immune response to pyelonephritis in the rabbit. J. infect. Dis. **128,** 195 (1973).
20. KUNIN, C. M., BEARD, M. V., HALMÁGYI, N. E.: Evidence for a common hapten associated with endotoxin fractions of *E. coli* and other Enterobacteriaceae. Proc. Soc. exp. Biol. (N. Y.) **111,** 160 (1964).
21. KUDO, K.: Common Antigentiter zur serologischen Diagose geeignet. Med. Trib. **8/9,** 1 (1973).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (3—4), pp 219—228 (1974)

DRUG-INDUCED MANIFESTATION OF HEREDITARY HEPATOPATHY

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Received September 9, 1974

Four cases are reported in which manifestation of a hitherto undetected enzymopathy has been provoked by drug treatment. The implications of genetic susceptibility to pharmacogenetic factors are discussed in the light of two cases of acute intermittent porphyria and two of Gilbert's syndrome.

Many hereditary diseases appear in clinically manifested forms. There are, however, hereditary metabolic errors of certain types which require some provocative factor for their manifestation. Drugs are a common source of provocative effects of this kind.

Investigations into the side effects of drugs [9, 10, 16] have thrown light on various genetic defects, the manifestation of which is connected with use of drugs.

Some of these defects occur as life-threatening complications, some others are merely indicators of a particular genetic constellation without any pathological or clinical involvement. The manner of transmission, including the monogenous or the multifactorial nature of the hereditary pattern, has been clarified in a number of genetic abnormalities. These side-effects of hereditary nature may be classified, according to the type of gene mutation, as follows:

1. modification of the chemical structure,
2. enzyme defect,
3. enzyme overproduction.

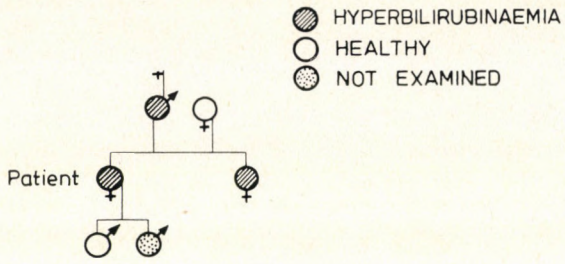
In the following four cases will be reported in which manifestation of the genetic defect was elicited by drugs. On the basis of Clarke's classification, two of the cases fall into the category of enzyme defects, two into that of enzyme overproduction.

Case 1. P. V., a 29 years old female patient had been healthy, had had two normal births and an abortion. She had then received oral contraceptive containing 2.5 mg noretynodrel and 0.1 mg mestranol. When one year on the pill, routine tests revealed indirect hyperbilirubinemia, and she was referred to our Department for investigations.

On admission she was well, in her family history there was no indication of haemolysis or of functional hyperbilirubinaemia. The liver was palpable

1.5 cm below the costal arch, it was moderately firm, of smooth surface and rounded border. The sclera was subicteric.

Laboratory findings: ESR 15 mm/hour; erythrocytes 4 500 000/cubmm; leukocytes 5400/cubmm; differential count: young form 2%, staff cells 2%,



	SE DI	BI INDI	ALP	GOT	GPT	BSP Tm	MENTHOL GLUCURONIDE
19.Nov. 1970.	NEG.	1,9mg %	2,6BU	22U	4U	5,9 mg/min	201 mg/5h
3.Febr. 1971.	NEG.	0,8mg %	2,8BU	29U	44U	10,0 mg/min	662 mg/5h

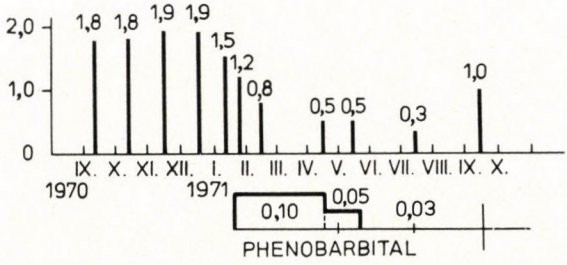


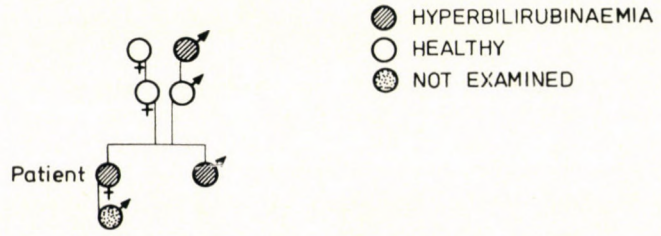
Fig. 1. Case 1.

neutrophils 58%, lymphocytes 32%, eosinophils 2%, monocytes 4%. Urine: spec. gravity: 1016, urobolinogen slightly increased, no other abnormality. Serum bilirubin 1.9 mg per 100 ml, no direct reaction; SGOT 22 U; SGPT 4 U; SALP 2.3 BU. Serum protein 7.63 g per 100 ml. Reticulocytes 8 in 1000. Osmotic resistance of RBC: normal; morphology of RBC: normal; Coombs test negative; serum iron: 182 μg per 100 ml; serum electrophoretic pattern: albumin 49%, α_1-globulin 6%, α_2-globulin 11%, β-globulin 14%, γ-globulin 21%. Cholescystography: normal filling of the gall bladder with no evidence of calculi; adequate contraction in response to grease meal. Liver biopsy: normal microscopic structure, fairly glycogen-rich parenchymal cells.

The foregoing results were exclusive of haemolysis as well as of any active disease of the liver parenchyma but raised the suspicion of Gilbert's syndrome.

(Fig. 1) The hereditary character of indirect hyperbilirubinaemia was fully borne out by the family studies. The result of the menthol loading test [15] revealed the disturbance of glucuronidation, and the value of BSP Tm [5] verified the diminished capability of anion excretion. The presence of Gilbert's syndrome was thus confirmed partly by way of exclusion, partly by the investigations directed at the transfer of bilirubin, and partly by the results of the family studies for serum bilirubin level.

Enzyme induction with phenobarbital [1, 6] resulted in complete normalisation of the findings. Fig. 1 shows the results.



	SE DI	BI INDI	GOT	GPT	ALP	MENTHOL GLUCURONIDE
9. March 1972.	NEG.	1,0 mg%	-	-	-	-
12. Apr. 1972.	NEG.	1,14	8 U	5 U	-	-
19. July 1972.	NEG.	2,16 mg%	10 IU	2 IU	-	-
Sept. 1972.	0,56 mg%	0,7 mg%	5 IU	4 IU	20,7 IU	160 mg/5^{h}

Fig. 2. Case 2

Case 2. The patient, K. I., was a 19 years old female. She had had one childbirth and an abortion and had then started taking oral contraceptive containing 1.0 mg ethynodiole diacetate and 0.05 mg ethynoloestradiol. After one month she found herself jaundiced and was referred to our Department.

On admission she was well. The liver was palpable 1.5 cm below the costal arch, it was soft, unsensitive to pressure, of rounded border. The sclera was subicteric.

Laboratory findings: ESR 10 mm/hour; erythrocytes 3 800 000/cubmm; leucocytes 4600/cubmm; Hb: 13.8 g per 100 ml; differential count: band forms 2%, neutrophils 66%, lymphocytes 30%, monocytes 2%. Urine: specific gravity 1030, urobilinogen slightly increased, no other abnormality. Serum bilirubin 1.26 mg per 100 ml, direct reacting bilirubin 0.56 mg per 100 ml; thymol turbidity 1.8 U; SGOT 5 IU; SGPT 4 IU; SALP 20 IU. Serum total protein 7.28 g per 100 ml. Reticulocytes 5 in 1000. Osmotic resistance and mor-

phology of RBC normal, Coombs test negative. Serum iron 114 μg per 100 ml. Serum electrophoretic pattern: albumin 66%, α_1-globulin 4%, α_2-globulin 5%, β-globulin 9%, γ-globulin 16%. Cholecystography showed good filling and adequate contraction of gall bladder with no evidence of calculi.

Liver biopsy: The liver cells had clear cytoplasm and moderate glycogen content. No inflammatory infiltration. In the unstained sections fine intracellular pigment granules were observed. The hereditary nature of disease was confirmed by the family study and by disturbance of bilirubin transfer with menthol loading. Withdrawal of the contraceptive without any other treatment resulted in complete normalisation in a few months (Fig. 2).

Case 3. S. E. a 27 years old female patient had had four miscarriages. In her adolescence she had undergone appendectomy a few days after the onset of non-acute symptoms. Histology of the surgical specimen had shown subacute appendicitis. Before admission, insomnia and restlessness regarded as non-organic had provided a source of exposure to various drugs of inductive properties. The present manifestations had been preceded by the regular use of chlordiazepoxide; she had also been taking glutethimid periodically. She had however, never experienced symptoms which might have been interpreted as a true attack of porphyria, and the family history was also negative in this respect. She was admitted to our Department in a fairly improved condition after an attack of acute porphyria. The liver was sightly enlarged, but no abnormality suggestive of organic disease was found. The neurological status was likewise negative.

Laboratory findings: ESR 20 mm/h; erythrocytes 4 200 000/cubmm; leukocytes 7800 /cubmm; Hb 12.9 g per 100 ml; differential count: staff forms 6%, granulocytes 78%, lymphocytes 16%. Platelet count 300 000. Urine dark red, specific gravity 1013, urobilinogen strongly positive, no other abnormality. Serum blirubin 0.45 mg per 100 ml; direct reacting bilirubin 0.24 mg per 100 ml; SGOT 24 IU; SGPT 12 IU; SALP 23 IU. Serum total protein 7.1 g per 100 ml. Reticulocytes 8 in 1000. Serum iron 119 μg per 100 ml. Serum electrophoretic pattern: albumin 54%, α_1-globulin 6%, α_2-globulin 13%, β-globulin 15%, γ-globulin 12%. Porphyrin excretion in 24 hr. urine: DALA: 50.3 mg/24 hr.; PBG: 89.5 mg/24 hr. uroporphyrin 4088 μg/24 hr.; coproporphyrin 683 μg/24 hr. Liver biopsy revealed a preserved microscopic structure. No fluorescence under Wood's light.

The symptoms and the presence of porphyrin precursors in the urine clearly pointed to an acute intermittent porphyria provoked by drugs [2].

Porphyria has no new causal therapy [13]. For the time being, carbohydrate diets offer the only reliable means for the suppression of ALA-synthetase [11]. Since such diet had been applied prior to admission, we placed her on allopurinol treatment which in laboratory animals as well as in humans had proved inhibitory to the microsomal enzyme systems [17], which are

the site of ALA-synthetase production, too. The drug was administered in doses of 100 mg, later of 300 mg daily. The results of treatment are seen in Figs 3,4. The precursors were measured by the ionexchange method of MAUZERALL and GRANICK, uro- and coproporphyrin by the extraction method of RIMINGTON (Figs 3, 4).

In view of the hereditary character of acute intermittent porphyria [14], screening of the family was done but this proved negative (Fig. 5). We have

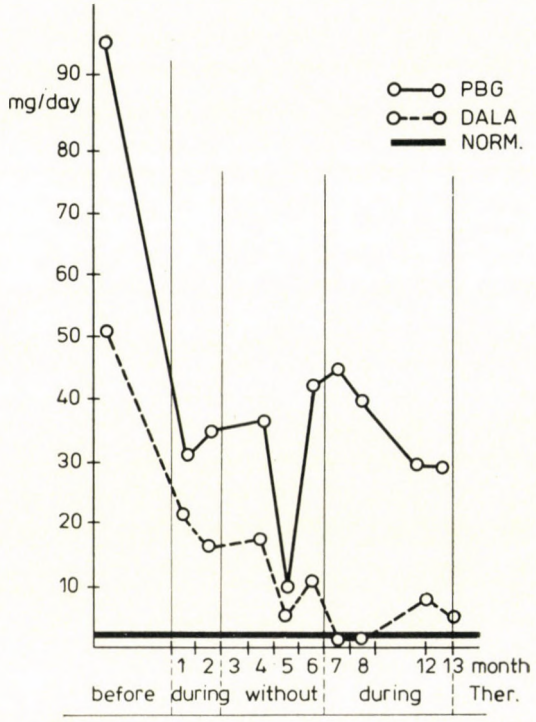


Fig. 3. Case 3. Response to therapy of porphyrin-precursor excretion

therefore performed a blood group study of the family. This proved that it was indeed a family in its true biological meaning. It was however remarkable that the patient represented an uncommon variant in respect of blood group heredity. She was, in fact, the Rh negative child of Rh positive parents, a constellation which presupposes both parents to be heterozygous for Rh. In this manner, the patient, in opposition to the other siblings had inherited two sets of c, d, e and was, unique in respect of porphyrin-excretion, and that of blood group.

Case 4. Sz. K., was a 26 years old female patient. She had had one childbirth two spontaneous and two induced abortions. She had had periodic colic and had been under observation repeatedly in different hospitals for symptoms ascribed to cystitis, still apart from a dilatation of the right renal pelvis and recurrent urinary infections, no abnormality had been found. Then she was admitted to a medical unit because of abdominal symptoms where acute intermittent porphyria was diagnosed and she was referred for treatment to

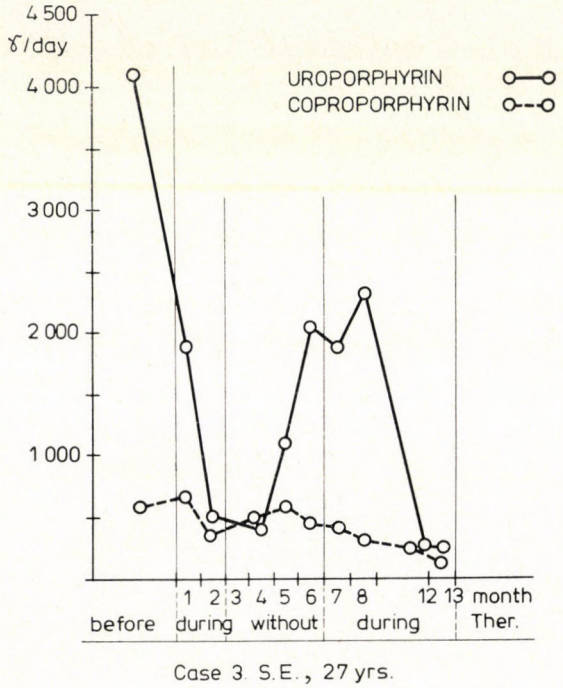


Fig. 4. Case 3. Response to therapy of excretion of porphyrin derivatives

our Department. We could trace back the manifestation of the condition to the use of sulphamethoxypyridazine administered for the urinary infection.

On admission her liver was slightly enlarged but not sensitive to pressure. No neurological or other abnormality was found.

Laboratory findings: ESR 17 mm/h; erythrocytes 3 400 000/cubmm; leukocytes 5600 /cubmm; Hb 12.6 g per 100 ml. Urine was dark red, specific gravity 1024, gave an intensive reaction for urobilinogen but was otherwise negative. Serum bilirubin 0.8 mg per 100 ml, no direct reaction. SGOT 31 IU; SGPT 15 IU; SALP 2.6 BU. Serum total protein 8.06 g per 100 ml. Reticulocytes 4 in 1000. Serum iron 130 μg per 100 ml. Serum electrophoretic pattern albumin 55%, α_1-globulin 4%, α_2-globulin 6%, β-globulin 16%, γ-globulin

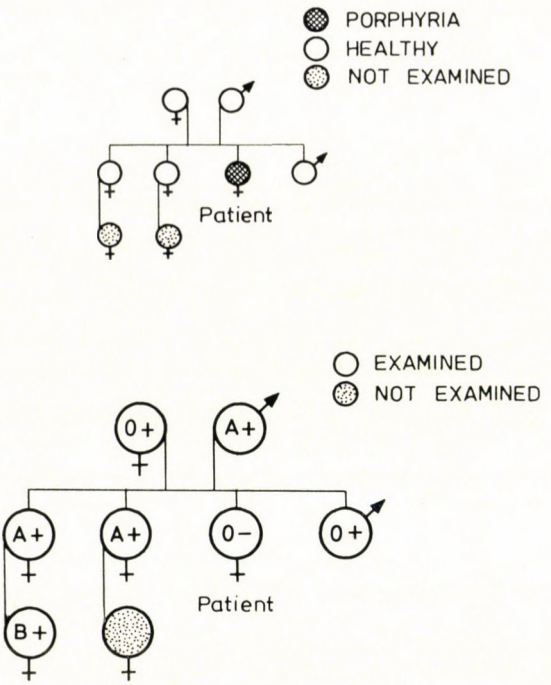


Fig. 5. Case 3. Family tree

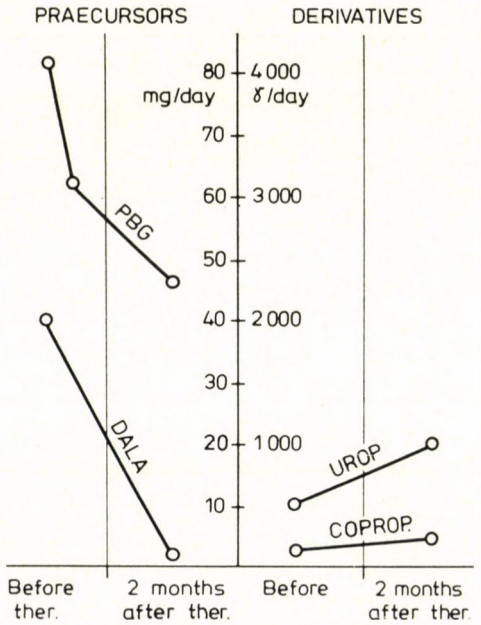


Fig. 6. Case 4. Response to therapy of porphyrin excretion

19%. Porphyrin excretion in 24 hr urine: DALA 40 mg, PBG 81.5 mg; uroporphyrin 504 μg, coproporphyrin 81.5 μg.

Liver biopsy: revealed in the parenchyma an intensive PAS-reaction as a sign of increased glycogen content. No hyperplasia of connective tissue or inflammatory infiltration was demonstrable.

Similarly as in the previous case, allopurinol was administered. In response to this, a fall in the excretion of precursors took place parallel with clinical improvement (Fig. 6).

Since the patient felt completely recovered we had to discharge her before having been able to arrange for the planned family screening.

Discussion

In the four presented cases, exposure to some drug was followed by side-effects of hereditary nature. It seems likely that, were this possibility always borne in mind, the hereditary character of manifestations of this kind might be confirmed more often in retrospect by directing the anamnesis at the previous use of drugs. The development of the pathological symptoms of our cases which appeared after commonly expected medication, can be well comprehended according to our present knowledge.

Crigler-Najjar's syndrome, a hereditary glucuronyl transferase deficiency is manifesting itself spontaneously in the neonatal period. Its prognosis, generally held to be unfavourable, has come to be regarded as dual, since two types of different therapeutic responsiveness may occur [9]. The symptoms of Gilbert's syndrome shows similarities, with the difference that it manifests in adolescence without any unfavourable prognosis, meaning no total enzyme deficiency. The presence of a mild unconjugated hyperbilirubinaemia is ascribed to an impairment of the glucuronide-conjugating capacity. Whether it is the partial deficiency of UDP-glucuronyltransferase alone which accounts for the symptoms or whether other mechanisms are also involved is still a controversial point [14]. It is certain, that the glucuronide production insufficient, in the physiology of which other enzymes are also involved [3]. The activity of these enzymes has been found to be hormone-dependent. It has been confirmed by animal studies that the majority of the natural as well as of the synthetic oestrogens are inhibitory to conjugation with glucuronide [6, 7]. A number of gestagens too have properties of this kind. Observations in humans on the above have been largely confined to the perinatal period [1]. Beta-glucuronidase is activated, too toward splitting the glucuronide by female sex-hormones [13].

The methods which have provided confirmation of Gilbert's syndrome have been described elsewhere [5, 8]. These methods, besides proving the dis-

turbance of the bilirubin transport, are also suitable indicators of the beneficial effect of phenobarbital induction.

While in Gilbert's syndrome, at least as far as the symptoms are concerned, enzyme induction is beneficial [12], in acute intermittent porphyria the effect of enzyme inducers is unfavourable [2, 11], since the overproduction of DALA-synthetase leads to an acute attack of porphyria. Despite various attempts in this field, reliable therapy of hepatic porphyria has yet to be found [13]. The number of our cases is too small to be conclusive in this respect. However, in a larger group of patients with acquired hepatic porphyria we have obtained similar results.

A point of interest in our first case of porphyria is the coincidence of the rare blood group variant with the only manifestation of inherited porphyria in the family. Joint hereditary transmission of blood group and of acute intermittent porphyria has not yet been reported in the literature. Our observation raises the question whether there is connection between the hereditary trend of blood groups and the manifestation of this inherited porphyria.

As a result of the increasing consumption of drugs the chances for the manifestation of hereditary drug side effects have also increased. While manifestation of Gilbert's syndrome or an increase in unconjugated hyperbilirubinaemia are regarded as mere cosmetic disorders, the inductive effect on a subject affected with porphyria involves life-threatening situations.

Our observations stress the part played by the genetic constellation in the side-effects of drugs. Even the most trivial pharmacotherapy involves hazards in genetically vulnerable individuals.

REFERENCES

1. Catz, C., Yaffe, S. J.: Amer. J. Dis. Child. **104,** 516 (1962).
2. D'Arcy, P. F., Griffin, J. P.: Iatrogenic diseases. Oxford University Press, London P88 (1972).
3. Dohrmann, R. E.: Beta-Glukuronidase. Springer Verlag, Berlin (1969) P. 36.
4. Fleischner, G., I. M. Arias: Amer. J. Med. **49,** 576 (1970).
5. Gógl, Á., T. Jávor: Acta physiol. Acad. Sci. hung. **39,** 188 (1971).
6. Hargreaves, T., R. F. Piper, Y. Cam: Nature New Biol. **110,** 243 (1971).
7. Hargreaves, T.: The Liver and Bile Metabolism. North-Holland Publishing Company, Amsterdam (1968) P. 349.
8. Horváth, T., Á. Gógl, G. Bücs, T. Jávor: Congress of Hungarian Pharmacological Society Budapest (1971).
9. Kalow, V.: Pharmacogenetics: Heredity and the Response to Drugs. Saunders, Philadelphia (1962).
10. Kuemmerle, H. P., E. R. Garrett, K. H. Spitzy: Klinische Pharmakologie und Pharmakotherapie. Urban und Schwarzenberg, München (1971) P. 93.
11. Rechcigl, M. jr.: Enzyme Synthesis and Degradation in Mammalian Systems. Ed.: Rechcigl, M., jr. Karger, Basel (1971) P. 270.
12. Roberts, R. J., G. L. Plaa: Biochem. Pharmacol. **16,** 827 (1967).
13. Róth, I.: Az orvostudomány aktuális problémái **3,** 120 (1969).

14. SCHMID, R.: The Porphyrias. The Metabolic Basis of Inherited Disease STANBURY, J. B., J. B. WYNGAARDEN, D. S. FREDERICKSON (eds.): (1972) P. 813. McGraw-Hill, New York.
15. SZABÓ, L., ÉBREY, P.: Orv. Hetil. **105,** 2023 (1964).
16. SZÓRÁDY, I.: Ther. hung. **18,** 63 (1970).
17. VESELL, E. S., G. T. PASSANANTI, F. E. GREENE: New Engl. J. Med. **283,** 1484 (1970).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (3—4), pp 229—239 (1974)

EFFECT OF ACUTE CHOLESTASIS ON HEPATIC CIRCULATION

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Received September 13, 1974

In dogs anaesthetized with pentobarbital, hepatic artery and portal vein blood flows were measured before and 6 hr after common bile duct ligation. With the rise of bile pressure, hepatic artery flow increased and resistance decreased. At the same time there was an increase in resistance and a decrease in flow in the portal venous system. Total hepatic blood flow was practically unchanged. It is suggested that increased hepatic arterial flow is the primary change in hepatic circulation. Accordingly, in bile stasis increased interstitial pressure causes arteriolar vasodilatation through a myogenic reaction triggered by decreased transmural pressure. The main cause of the decrease of portal flow is the arteriolar constriction in the prehepatic splanchnic bed elicited probably by the increased portal resistance.

Chronic total extrahepatic biliary obstruction decreases hepatic blood flow [2, 3] deranges the distribution of flow in the intrahepatic arteries and veins [8, 10, 18] and increases the collateral arterial blood supply of the liver [19]. Surprisingly little is known on the acute effects of biliary stasis on hepatic circulation. In acute extrahepatic obstruction the liver is enlarged, the biliary ducts and the sinusoids are dilated, and there are marked changes in the fluid matrix of the liver. All this might be expected to influence hepatic blood flow, especially in the low-pressure portal system.

In a previous study [26] it was shown that acute occlusion of the extrahepatic bile passages in dogs decreases hepatic blood flow and increases portal venous pressure. These investigations were, however, done with the sulphobromphthalein extraction technique which is notoriously unreliable in the presence of biliary obstruction. In another study [20] in dogs the hepatic venous outflow was directly measured after occlusion of the thoracic inferior vena cava and diversion of the blood from the abdominal caval vein. By this technique, a significant decrease of hepatic blood flow was observed 1 hour after occlusion of the common duct. After 1 week of bile stasis, blood flow was normal again. Measured by the ^{133}Xe wash-out technique, overall blood supply to the intact liver following bile duct ligation did not appear to decrease before 72 hours in rats and before the 5th day in dogs [16]. Recently, more advanced methods have become available for the measurement of liver blood flow in experimental animals. It was therefore decided to reinvestigate the effect of acute cholestasis on hepatic circulation.

Material and method

The experiments were done on 10 mongrel dogs of either sex with an average body weigh of 16.9 kg (12—23 kg) in pentobarbital anaesthesia (30 mg/kg). The dogs had endotracheal tubes in place and were ventilated, if necessary, mechanically with a piston respirator. A midline laparatomy was performed and a small mesenteric vein was cannulated with a fine polyethylene catheter. Its tip was passed into the portal vein. The cystic duct was ligated and the common duct transected and cannulated with a Teflon tube. The common hepatic artery and the portal vein were exposed. Electromagnetic flow probes were placed on the vessels. Mechanical zero points of the flow probes were checked at the begining and at the end of the experiments.

After conclusion of the surgical intervention, the abdomen was closed with sutures. The flow probes were connected to a Nycotron electromagnetic flow meter. Portal venous and arterial (femoral artery) pressures were measured with Statham strain gauges connected to Hellige electronic integrators. The zero points of the manometers were set 5 cm above the level of the operating table. Flows and pressures were registered by a multichannel direct writing recorder.

Following a one-hour postoperative interval, control measurements of blood flows and pressures were obtained and the common duct was occluded by connecting the end of the cannula directly to a pressure transducer. Haemodynamic parameters were estimated every 30 min for 6 hr.

The results were calculated in terms of the mean $\pm$ standard error. Statistical significance of the changes was assessed with Student's t test applied to the per cent differences between the control and observed values.

For technical reasons, hepatic venous and sinusoidal *i.e.* hepatic vein "wedge" pressures were not measured. Accordingly, intrahepatic flow resistances could not be determined. Total resistance to hepatic artery and portal venous inflow were calculated by dividing portal venous and hepatic artery pressures with the respective blood flows. Preportal resistance in the mesenteric vascular bed, *i.e.* splanchnic arteriolar resistance, was calculated by dividing the difference of the arterial and portal venous pressures by portal venous flow. All values were expressed in peripheral resistance units (PRU).

In 3 additional dogs the same surgical procedures were performed but the common duct was not occluded. In these sham operated controls the same parameters as in the previous group were recorded continuously for 6 hr.

Results

In the 10 dogs, the control value for hepatic artery flow was 177 ± 27 ml/min, and for portal vein flow, 439 ± 80 ml/min, giving a hepatic inflow of 616 ± 109 ml/min or 37.2 ± 3.0 ml/min/kg body weight (Table I and Fig. 1). This means that the hepatic artery contributed 29%, and the portal vein, 71%, to the total hepatic blood flow. These results were in good agreement with the values published in the literature and obtained by similar techniques [12]. Portal venous pressure was 7.7 ± 0.7 mm Hg. Pressure in the common duct was before its cannulation 5.6 ± 0.7 mm Hg. Arterial blood pressure was 124 ± 5 mm Hg and it was kept constant up to the end of the experiment.

After occlusion of the common duct, its end pressure rose rapidly, attaining 20 mm Hg on the average in 1 to 1 1/2 hour (Fig. 1). The rise of biliary pressure was followed by an increase in hepatic artery flow attaining a maximum 95% increase in 2 1/2 to 3 hours. At the same time a decrease of portal vein flow was noted. This change was, however, slower and progressed practically up to the end of the experiment. By this time, portal flow has decreased

by some 50%. As a result of these opposing changes, total hepatic inflow remained essentially constant. An appreciable decrease of total hepatic blood flow occurred only in the last half hour of the experiment (—13% $p > 20\%$). Portal vein pressure rose in most animals; accordingly, there was a substantial increase of total portal resistance: from 1.75 ± 0.40 PRU to 6.06 ± 2.73 PRU at the end of the experiments. On the other hand, arterial resistance decreased

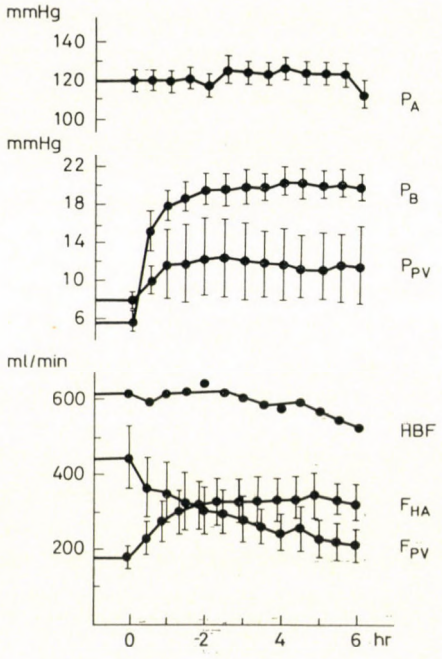

Fig. 1. Effect of common duct occlusion on liver circulation. After ligation of the common duct and of the cystic duct (at 0 hour), biliary pressure (P_B) rises, and there is a small increase in portal venous (P_{PV}) pressure. Hepatic artery flow (F_{HA}) increases and portal venous flow (F_{PV}) decreases

from 51 ± 16 to 26 ± 5 PRU (Fig. 2 and Table II), *i.e.* arterial resistance diminished by 45% and portal vein resistance was more than doubled. Consequently, *e.g.* at the end of the 5th hour after common duct occlusion, total hepatic inflow was 565 ml/min, and to this the hepatic artery contributed 339 ml (60%) and the portal vein, 226 ml/min (40%). Thus, at this stage, at nearly stable total hepatic blood flow, there was an inversion of the relative contributions of the two vessels. Finally, resistance in the prehepatic splanchnic vascular bed, *i.e.* splanchnic arteriolar resistance, increased from 24.5 ± 3.0 to 53.1 ± 10.8 PRU in 5 h.

As expected, there was a significant correlation between the increase in common duct pressure and the changes of arterial and portal vein flows

Table I

Statistical analysis of the changes in liver circulation after common duct

Hours after occlusion		0.5	1.0	1.5
Total hepatic flow diff., per cent	$\bar{X}$	−2.2	+1.4	+2.6
Average control value	S±	3.4	6.3	7.2
616 ± 109 ml/min	P	>70%	>80%	>80%
Hepatic artery flow diff., per cent	$\bar{X}$	+29	+47	+60
Average control value	S±	6.2	11.7	14.0
177 ± 27 ml/min	P	<1%	<1%	<1%
Portal vein flow diff., per cent	$\bar{X}$	−13	−17	−21
Average control value	S±	3.2	5.1	6.2
439 ± 80 ml/min	P	<1%	<1%	<1%
Portal vein pressure diff., mm Hg	$\bar{X}$	+2.1	+3.9	+3.3
Average control value	S±	− 0.79	1.07	1.15
7.7 ± 0.7 mm Hg	P	>5%	<1%	<2%

($r_{HA} = 0.615$, $p < 0.1\%$: $r_{PV} = -0.665 < 0.1\%$). No correlation was found between the changes of portal vein pressure and flow ($r = -0.166$; $p > 10\%$). There was a wide scatter even if the average pressure values were plotted against the average flows observed at the same time. The same method of plotting revealed that the relationship between bile duct pressure and blood flow in the hepatic artery and the portal vein was not linear. This was obvious, especially in the case of portal flow. In the higher ranges of common duct pressure, small changes apparently produce considerable drops of portal flow (Fig. 3). The time factor should however not be neglected. The interpretation

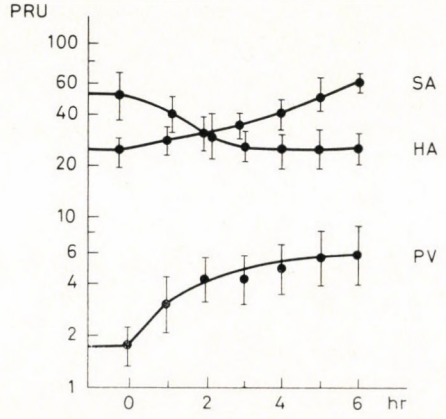

Fig. 2. Changes in vascular resistances after common duct occlusion. SA: prehepatic splanchnic arteriolar resistance; HA: hepatic artery resistance; PV: portal inflow resistance

occlusion (n = 10)

2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
+1.8	0	+1.2	−3.1	−3.4	−5.1	−5.2	−6.3	−12.9
7.9	6.4	7.6	7.0	8.7	8.9	9.7	10.8	9.5
>80%		>90%	>70%	>70%	>60%	>70%	>60%	>20%
+71	+79	+84	+87	+89	+90	+95	+93	+91
13.6	12.8	10.8	13.0	12.7	14.4	18.0	15.7	16.8
<0.1%	<0.1%	<0.1%	<0.1%	<0.1%	<0.1%	<0.1%	<0.1%	<0.1
−25	−26	−30	−34	−39	−44	−48	−50	−51
7.5	4.5	5.6	7.1	6.6	6.6	7.5	8.1	8.9
<2%	<0.1%	<0.1%	<1%	<0.1%	<0.1%	<0.1%	<0.1%	<0.1%
+4.4	+4.5	+4.3	+3.8	+4.1	+3.1	+3.2	+3.9	+3.7
1.43	1.28	1.21	1.09	1.24	1.01	1.04	1.35	1.51
<2%	<1%	<1%	<1%	<1%	<2%	<2%	<2%	<2%

* Diff. per cent: percentual changes of blood flow or circulation resistance $= 100 \left(\frac{\text{observed}}{\text{control}} - 1 \right)$

that a sustained high pressure in the common duct leads to a progressive decrease of portal blood flow, would be clearly more in accordance with the experimental observations.

A correlation should be expected between the changes of portal flow or resistance and of arterial flow and resistance. When all observed values were pooled, no linear correlation was found (for flow, $r = 0.120$, $p > 10\%$; for resistance, $r = -0.168$, $p > 10\%$). In Fig. 4 average hepatic artery flows are

Table II

Statistical analysis of the changes in hepatic vascular resistances (n = 10)

Hours after common duct occlusion		1	2	3	4	5	6
Hepatic artery resistance diff. per cent	$\bar{X}$	−30	−42	−44	−43	−46	−43
Average control value	S±	6.0	3.7	3.2	4.6	5.0	3.6
51 ± 16 PRU	P	<1%	<0.1%	<0.1%	<0.1%	<0.1%	<0.1%
Portal vein resistance diff. per cent	$\bar{X}$	+85	+161	+157	+186	+213	+204
Average control value	S±	32	77	53	59	80	70
1.75 ± 0.40 PRU	P	<5%	<5%	<2%	<2%	<5%	<5%
Prehepatic splanchnic resistance diff. per cent	$\bar{X}$	+23	+47	+55	+84	+126	+150
Average control value	S±	8.1	19.4	15.5	22.2	29.8	32.8
24.5 ± 3.0 PRU	P	<5%	<5%	<1%	<1%	<1%	<0.1%

plotted against average portal vein flows. It can be seen that the relationship between the two sets of values is a complex one. With declining portal flow, arterial flow rises at first steeply, attaining a maximum above which there is little change. In the first 2 1/2 hours of the experiment, the regression curve

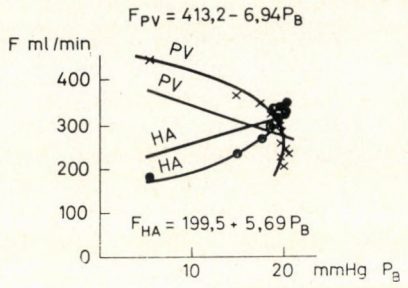

Fig. 3. Relationship between biliary pressure (P_B), and portal vein (F_{PV}) and hepatic artery (F_{HA}) flow, after common duct occlusion. A significant correlation was found between biliary pressure and portal vein blood flow, as well as between biliary pressure and hepatic artery flow. The straight lines in the figure are the corresponding regression lines (with the linear regression equations). The dots and crosses are values obtained in the experiments (averages of all measurements made at a given point of time). The curves connecting these points show that the relationship between flow and pressure changes is not linear

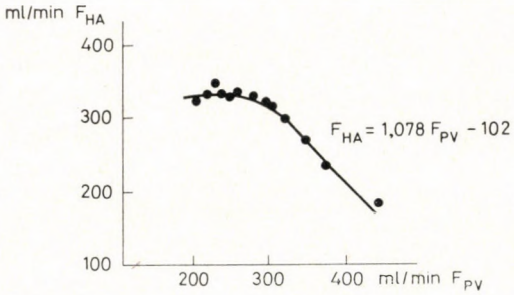

Fig. 4. Relationship of average hepatic artery flow (F_{HA}) and portal venous flow (F_{PV}). The regression equation refers to the ascending part of the curve

is practically linear. For this steeply ascending line a high correlation coefficient was found ($\bar{r}' = -0.991$, $p < 0.1\%$). For the second part of the curve the coefficient of correlation was only -0.455 ($p > 10\%$). The relationship between common duct pressure, hepatic artery flow and portal vein flow was examined in the first 2 1/2 hours of the experiments (*i.e.* at the nearly straight parts of the respective curves) also by multiple variable regression analysis [24]. The partial correlation coefficients suggested that at constant bile duct pressure there would still remain a significant correlation between hepatic artery and portal vein blood flows ($r = -0.933$, $p < 1\%$). At constant arterial

flow, the correlation between portal flow and bile pressure would be $\bar{r} = -0.812$ ($p < 5\%$), but at constant portal vein flow there would be no significant correlation between bile duct pressure and hepatic artery flow ($\bar{r} = 0.474$; $p > 20\%$). Dividing the standardized partial regression coefficients with each other it was found that the changes of portal vein flow had about 2.5 times more influence on the variations of hepatic artery flow than had the changes in common duct pressure. The results of a partial correlation coefficient analysis should be interpreted with caution, but it can safely be concluded that the connection between the changes of hepatic artery and portal vein flows merits further analysis. The percentual changes of portal vein flow were plotted in Fig. 5 against the percentual changes of hepatic artery flow. The resulting curve is of the saturation type, *i.e.* with the decrease of portal vein flow hepatic artery flow at first rises nearly in a linear fashion, attaining a maximum after which it does not change. A similar curve was obtained, when the changes of hepatic artery resistance were plotted against the changes of portal vein resistance (Fig. 5).

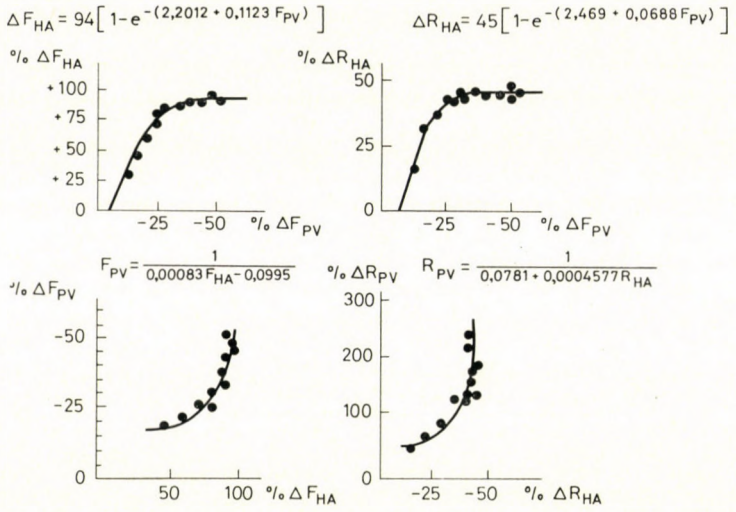

Fig. 5. Relationship of hepatic artery and portal vein flow and resistance changes after common duct occlusion. In the upper row, the percentual changes of hepatic artery flow ($\triangle F_{HA}$) and resistance ($\triangle R_{HA}$) are plotted against the changes of portal vein flow ($\triangle F_{PV}$). Saturation type regression curves are obtained. In the lower row, the changes of portal vein flow ($\triangle F_{PV}$) and resistance ($\triangle R_{PV}$) are plotted against the changes in hepatic artery flow ($\triangle F_{HA}$) and hepatic artery resistance ($\triangle R_{HA}$) respectively. In both instances hyperbolic regression curves were obtained

Finally, it was observed that when portal venous flow decreases, resistance in the prehepatic splanchnic bed rises markedly. Actually, an exponential relation was found between the fall of portal vein flow (or increase in portal

vascular resistance) and the increase in prehepatic splanchnic resistance (Fig. 6).

In the 3 sham operated animals, the measured parameters did not change significantly during the 6-hour observation period.

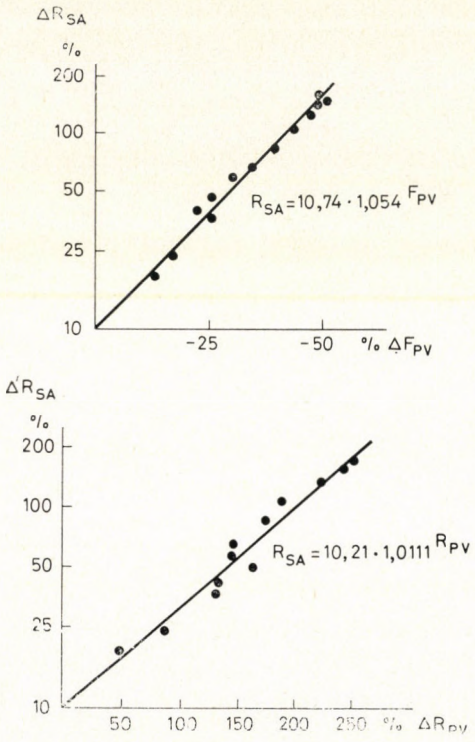

Fig. 6. Regression between changes in portal vein flow ($\triangle F_{PV}$) or resistance ($\triangle R_{PV}$) and the changes of prehepatic mesenteric arteriolar resistance (R_{SA}) after common duct occlusion. Note the exponential character of the regression curves and equations

Discussion

Occlusion of the common duct and the consequent increase in biliary pressure were found to cause a marked increase in hepatic artery flow, a reduction of portal vein flow and — at least during the 6 hr observation period — little change in total hepatic blood flow. These changes might be explained by the increase in the resistance to portal flow produced by the dilatation of the bile canaliculi and expansion of the extravascular fluid space in the liver, with a consecutive increase in interstitial fluid pressure affecting the flow of blood in the sinusoids. The increase of hepatic artery flow could be a secondary consequence of the decreased portal flow [1, 11, 17, 22].

Some observations made in the course of the present experiments have not supported the above assumption. Actually, in 2 of the 10 animals, portal venous pressure did not rise significantly after occlusion of the common duct, nor was there a change in portal blood flow in one of them. The hepatic artery flow increased nevertheless. The impression was also gained, that in most animals the increase in hepatic artery flow precedes the decrease in portal flow. Consequently, the possibility must be envisaged that the primary change in hepatic circulation is a rise of hepatic artery flow, caused by a decrease of hepatic artery resistance. The vascular reactions are not abolished by atropin or by blocking the alpha and beta receptors [25]. On the other hand, according to the myogenic theory of blood flow autoregulation, a decrease in transmural pressure across the arteriolar wall should lead to a vasodilatation. Actually, dilatation of the bile canaliculi and extravasation of bile into the interstitial fluid must raise the tissue pressure in the liver, decreasing by this the difference between the luminal and extraluminal arteriolar pressures. The arteriolar vasodilatation may raise the sinusoidal pressure and consequently cause a further increase in the portal flow resistance which is already affected by the elevated tissue pressure.

If an arteriolar vasodilatation is the primary change in hepatic circulation, then the changes of portal venous flow and resistance should be examined as a function of hepatic artery flow and resistance changes. With hepatic artery flow or resistance as an independent variable, a hyperbolic regression curve is obtained (Fig. 5), i.e. the reciprocals of the changes in portal vein flow and resistance are in an inverse linear correlation with the changes of hepatic artery flow and resistance.

The interaction between the two vascular systems in the liver and the autoregulation of hepatic blood flow is a disputed question. In early studies [4, 6, 17], corroborated by subsequent investigations [7, 9, 21, 23] a reciprocity was found between hepatic artery flow and portal vein flow. Observations made on preparations where the hepatic artery was not perfused showed that a decrease of portal pressure or flow contistently led to an increase in hepatic artery flow [13, 23, 27]. The changes in hepatic artery flow or pressure seem to produce inconsistent effects on portal pressure and flow [65, 9, 13, 15 27]. The liver accounts, however, for only about 10% of the total resistance in the splanchnic vascular bed. Accordingly, changes in hepatic resistance are unlikely to produce major changes in portal blood flow. There is, however, evidence for the increased mesenterial venous pressure to produce, at least in the intestines, a marked rise in total vascular resistance [14]. In the present experiments it was found that the increase in portal inflow resistance causes an exponential increase in prehepatic splanchnic resistance. This behaviour would be consistent with a myogenic autoregulation of blood flow in the intestines and possibly in some other organs supplied by the mesenteric vessels.

The observation that at changing hepatic artery and portal vein flows total hepatic blood flow remains practically constant is quite remarkable. The assumption of an interaction between the two hepatic circulations rests nevertheless mainly on the results of regression analyses, *i.e.* on indirect evidences. The possibility must therefore be envisaged that after common duct obstruction the changes in hepatic artery and portal vein flows are not interrelated, but are both caused independently by changes in bile pressure. The basic conclusions concerning the mechanism underlying the observed phenomenon would, however, not be affected by it. The main factor would remain the increase of interstitial tissue pressure reducing transmural hepatic arteriolar pressure and increasing portal inflow resistance. By these changes an arteriolar dilatation is triggered in the liver and a vasoconstriction in the mesenterial vascular bed.

REFERENCES

1. ANDREWS, W. H. H., FIELD, C. D.: An investigation of intra-hepatic circulation by means of an indicator-dilution technique. J. Physiol. (Lond.) **182,** 50 (1966).
2. ARONSEN, K. F.: Late effects of biliary stasis on the effective liver blood flow. Acta Chir. scand. **134,** 278—281 (1968).
3. ARONSEN, K. F., NYLANDER, G., OBISSON, E. G.: Liver blood flow studies during and after various periods of total biliary obstruction in the dog. Acta Chir. scand. **135,** 55—59 (1969).
4. BAUER, W. H., DALZ, H., POULSON, T., RICHARDS, D. W.: The control of circulation through the liver. J. Physiol. (Lond.) **74,** 343—375 (1932).
5. BOLLMANN, J. L., KHATTAB, M., THORS, M., GRINDLAY, R. & J. H.: Experimentally produced alterations of hepatic blood flow. Arch. Surg. **66,** 562—569 (1953).
6. BURTON-OPITZ, R.: The vascularity of the liver: the influence of the portal blood-flow upon the flow in the hepatic artery. Quart. J. exp. Physiol. **4,** 93—102 (1911).
7. COHN, R., KOUNTZ, S.: Factors influencing control of arterial circulation in the liver of the dog. Amer. J. Physiol. **205,** 1260—1264 (1963).
8. DEL RIO LOZANO, I., ANDREWS, W. H. H.: Changes in the hepatic vascular pattern that follow ligation of the common bile duct in rabbits. J. Path. Bact. **90,** 472—477 (1965).
9. GINSBURG, M., GRAYSON, G.: Factors controlling liver blood flow in the rat. J. Physiol. (Lond.) **123,** 574—602 (1954).
10. GLIEDMAN, M. L., GIRARDET, R. E., SCHWARTZ, A., RYZOFF, R., LERNER, B., KARLSON, K. E.: Hepatic vascular anatomy and manometry in experimental biliary obstruction and ascites. Surg. Gynec. Obstet. **119,** 749—757 (1964).
11. GRAYSON, J., MENDEL, D.: Observations on the intrahepatic flow interactions of the hepatic artery and portal vein. J. Physiol. (Lond.) **139,** 167—177 (1957).
12. GREENWAY, C. V., STARK, R. D.: Hepatic vascular bed. Physiol. Rev. **51,** 23—65 (1971).
13. HANSON, K. M., JOHNSON, P. C.: Local control of hepatic arterial and portal venous flow in the dog. Amer. J. Physiol. **221,** 712—720 (1966).
14. JOHNSON, P. C.: Origin, localization and homeostatic significance of autoregulation in the intestine. Circulat. Res. **15,** I-125-I-232 (1964).
15. KOCK, N. G., HAHNLOSER, P., RODING, B., SCHENK, W. G.: Interaction between portal venous and hepatic arterial blood flow. An experimental study in the dog. Surgery **72,** 414—419 (1972).
16. LUTZINA, A., BROWN, H., BROWN, M. E., MACDERMOTT, W. V.: Hepatic blood flow alterations following common bile duct ligation. Surg. Forum **19,** 340—341 (1958).
17. MACLEOD, J. J. R., PEARCE, R. G.: The outflow of blood from the liver as affected by variation in the condition of the portal vein and hepatic artery. Amer. J. Physiol. **25,** 87—105 (1914).
18. OHLSSON, G. E.: The arterial circulation in the liver after total biliary obstruction in dogs. Acta Chir. scand. **138,** 51—58 (1972).

19. POPPER, H., JEFFERSON, N. C., NECHELES, H.: Survival of dogs after partial or total devascularization of the liver. Ann. Surg. **140,** 93—99 (1954).
20. SAKODA, K., ATIK, M.: Influence of common bile duct ligation on hepatic blood flow. Amer. Surg. **36,** 731—736 (1970).
21. SANCETTA, S. M.: Dynamic and neurogenic factors determining the hepatic arterial flow after portal occlusion. Circulat. Res. **1,** 414—418 (1953).
22. SASKIN, S., ESSEX, H. E., HERRICK, J. F., MANN, F. C.: The mechanism of regulation of the blood sugar by the liver. Amer. J. Physiol. **124,** 558—567 (1938).
23. SCHENK, W. G., jr., MCDONALD, J. C., MCDONALD, K., TRAPANAS, T.: Direct measurement of hepatic blood flow in surgical patients: With related observations on hepatic flow dynamics in experimental animals. Ann. Surg. **156,** 462—471 (1962).
24. SVÁB, J.: Biometriai módszerek a kutatásban. Mezőgazdasági Kiadó, Budapest 1973.
25. SZABÓ, G., JAKAB, F., MAGYAR, Z.: Mechanism of the effect of increased common bile duct pressure on liver circulation. Acta Med. Acad. Sci. Hung. **31,** 243—252 (1975).
26. SZABÓ, G., MAGYAR, Z.: Die Wirkung der experimentellen Okklusion der Gallengänge auf den Leberkreislauf: Z. ges. exp. Med. **137,** 163—169 (1963).
27. TAKEUCHI, J., KUBE, T., TONE, T., TAKADA, A., KITAIGAWA, T., YOSHIDA, H.: Autoregulation and interaction between two vascular systems in dog liver. J. appl. Physiol. **27,** 77—82 (1969).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (3—4), pp 241—250 (1974)

THE MECHANISM OF THE EFFECT OF INCREASED BILIARY PRESSURE ON HEPATIC CIRCULATION

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Received September 26, 1974

The sudden increase to 40 mm Hg in common duct pressure causes in the dog an immediate 39% increase in hepatic arterial blood flow. At the same time, portal venous decreases by 9.5%. If biliary pressure is raised gradually, hepatic arterial flow changes at first only little but at a critical pressure between 20 and 35 mm Hg there is a sudden and nearly maximum increase in flow. The arterial reaction is not abolished by alpha and beta blocking agents or by atropine. Changes in the composition of the fluid distending the biliary passages do not influence the intensity of the reaction but may alter its course. The increase in hepatic arterial flow is caused by a myogenic arteriolar vasodilatation triggered by the decrease in effective vascular transmural pressure.

When biliary pressure rises after the occlusion of the common duct, blood flow in the hepatic artery increases and in the portal vein decreases. As a result of these opposite changes, total hepatic blood flow remains practically unaltered [8]. These observations were consistent with the assumption that the increase in hepatic artery flow is the primary change. The hypothesis was advanced that in biliary stasis, increased interstitial pressure causes an arteriolar vasodilatation through a myogenic reaction triggered by the decreased transmural pressure. The present investigations were designed mainly to provide experimental evidence for the myogenic origin of this arteriolar reaction and to offer additional informations concerning its mechanism.

Material and methods

The experiments were done on mongrel dogs of both sexes in general pentobarbital (30 mg/kg) anaesthesia. A midline laparatomy was performed and a fine polyethylene catheter was introduced through a small mesenteric branch into the portal vein. The hepatic artery and the portal vein were exposed. Electromagnetic flow probes were placed on the vessels. The cystic duct was ligated and the common duct transected and cannulated with a teflon tube.

After the surgical manipulations the abdomen was closed with sutures. The cannula introduced into the common duct was connected through a T-tube to a pressure transducer and to a fluid reservoir containing physiological saline, homologous serum or 6% dextran solution. By raising the reservoir, pressure in the common duct could be adjusted to a predetermined level. The flow probes on the hepatic artery and the portal vein were connected to a Nycotron electromagnetic flowmeter. Portal venous and systemic arterial (femoral artery) pressures were measured with Statham strain gauges connected to Hellige electronic integrators. The zero point of the manometers was set 5 cm above the level of the operating table. The flows and pressures were registered with a multichannel direct writing recorder. The results reported in the Tables and in the text are means $\pm$ standard error of the mean.

Results

Effect of an abrupt increase in common duct pressure

As a first step, the animals were tested for their reaction to a sudden increase of common duct pressure. For this purpose the cannula in the duct was connected with the fluid reservoir. By raising the container, a pressure of 40 mm Hg was applied to the duct. Among 22 examined animals, in 20 hepatic

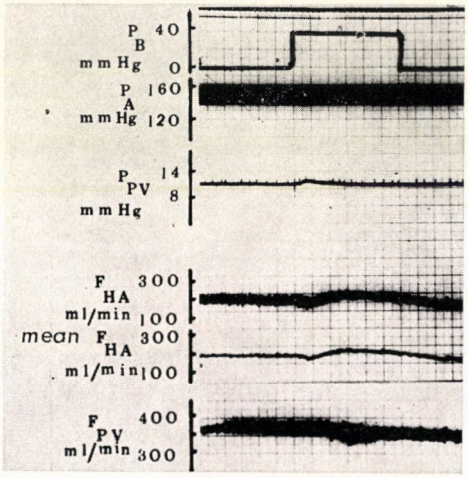

Fig. 1. Effect of a sudden increase of bile pressure (P_B) on arterial (P_A) and portal venous (P_{PV}) blood pressure, hepatic arterial (F_{HA}) and portal venous (F_{PV}) blood flow. (3 big squares = 10 min)

artery flow increased immediately while in 2 it remained unchanged. Initial hepatic artery flow was 218 ± 6 ml/min, this increased to 301 ± 9 ml/min, or by $39.3 \pm 4.3\%$. The reaction could be reproduced at will. When it was repeated in each animal 5 to 10 times, the arterial dilatation was always identical. The reaction of the portal vein was tested in 16 animals. In 5 there was no change in portal venous flow (31%), in 11 animals the flow decreased. The decrease was slight, more than 20% in a single instance only and the average decrease was 9.5%. No correlation was found between the changes of arterial and portal venous flows ($r = -0.120$; $p > 10\%$.) The results obtained in 14 experiments, where hepatic arterial and portal venous flows were measured simultaneously, are shown in Table I (the 2 animals in which neither of the flows displayed a change, were discarded). It can be seen that total hepatic inflow, calculated by adding the hepatic artery and portal vein flows, did not decrease in these experiments, but even showed a small, not significant, increase. The contribution of the hepatic artery to total hepatic blood flow increased from 31% to

41%. Control total hepatic blood flow was in this group 758±50 ml/min, or 39.0±3.1 ml/min/kg.

The changes in portal venous pressure were inconsistent. An increase was observed in 10 out of 22 experiments, in the remaining animals it did not change or even decreased slightly (Table I). Systemic arterial mean blood pressure was 133±5 mm Hg and did not change during the experiments. Accordingly, the increase in arterial flow was due to a decrease of vascular resistance.

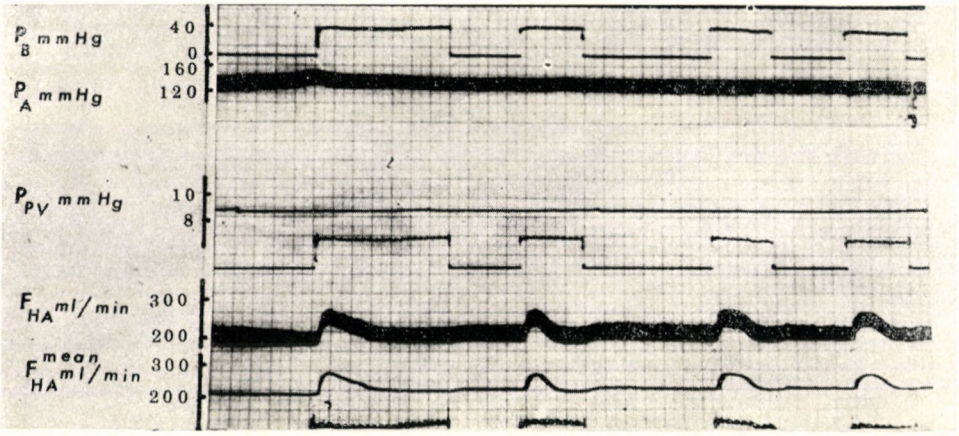

Fig. 2. Effect of repeated increases of biliary pressure (P_B) on arterial (P_A) and portal venous (P_{PV}) pressure and hepatic arterial flow (F_{HA}) (Biliary pressure was increased by infusion of 6% dextran solution into the common duct.) (3 big squares = 10 min)

Table I

Effects of an abrupt maximum increase of common duct pressure

Biliary pressure		0 mm Hg	40 mm Hg	Difference, per cent
Hepatic artery flow ml/min	$\bar{X}$	241	328	+38.1
(N = 14)	S±	23	33	6.2
	p%			<0.1
Portal vein flow ml/min	$\bar{X}$	517	468	−9.5
(N = 14)	S±	47	44	1.8
	p%			<0.1
Total hepatic blood flow ml/min	$\bar{X}$	758	796	+5.0
(N = 14)	S±	50	46	3.3
	p%			>10
Portal vein pressure mmHg	$\bar{X}$	9.6	10.3	+0.7
(N = 22)	S±	0.7	0.6	0.4
	p%			>10

The above experiments showed that the most constant and marked consequence of elevated biliary pressure is an increase in hepatic arterial blood flow.

Gradual increase of common duct pressure

In 10 dogs, biliary pressure was raised gradually by increments of 5 mm Hg at 5 minute intervals. At pressures below 20 mm Hg, bile was seen to flow into the cannula. If the container was raised further, between 20 and 35 mm Hg the direction of flow was inverted and fluid was beginning to flow from the

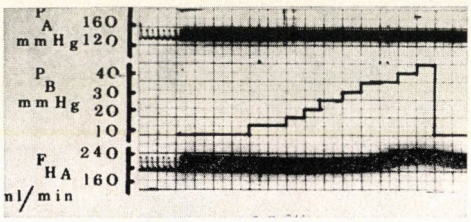

Fig. 3. Effect of a gradual increase of biliary pressure (P_B) on arterial pressure (P_A) and hepatic arterial flow (F_{HA}). (3 big squares = 10 min)

reservoir into the common duct. On raising the infusion pressure further, the rate of flow increased, reaching 1.0—2.5 ml/min at pressures above 40 mm Hg.

At pressures under 20 mm Hg there was no, or only a slight, change in hepatic arterial flow. A sudden increase of flow was observed between 20 and 35 mm Hg. Above 35 mm Hg there was again very little or no change (Table II). If the changes of hepatic artery flow are plotted against common duct pressure, a typical sigmoid or logistic curve is obtained (Fig. 4). The relation between pressure and flow changes is expressed by the regression equation

$$\triangle F_{HA} = 36 \frac{1}{1 + e^{4.887 - 0.198 \triangle P_B}}$$

Table II

Effect of increased common duct pressure on hepatic arterial blood flow (n = 10)

Biliary pressure, mm Hg		0	10	15	20	25	30	35	40
F_{HA} ml/min	$\bar{X}$	234	235	239	250	281	297	301	304
	S ±	36	35	34	34	42	36	44	43
$\triangle F_{HA}$ per cent	$\bar{X}$		2.0	5.0	8.7	20.3	27.9	31.8	33.7
	S ±		1.6	3.8	4.5	7.4	6.6	4.5	4.0

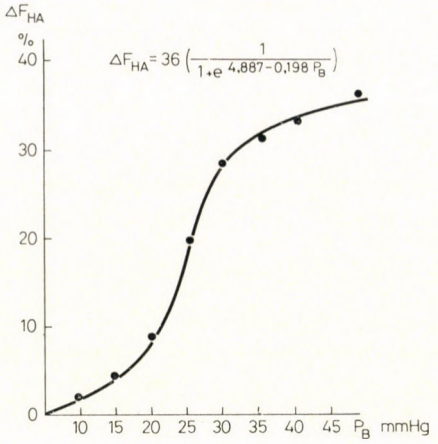

Fig. 4. Regression between biliary pressure (P_B) and the changes of hepatic arterial flow (ΔF_{HA})

Influence of the composition of the infused fluid

To test the hypothesis that the observed phenomenon was due to changes in interstitial fluid pressure rather than to the distension of the biliary ducts, in 11 animals 6% dextran solution and in 3 animals dog serum was infused instead of physiological saline. In these experiments, at 40 mm Hg common duct pressure hepatic arterial flow increased from 238±9 ml/min to 313± 11 ml/min. The average increase was 36.2±0.9%. This value was not significantly different from the average increase observed in the experiments with saline infusion (39.3±4.3%). There was, however, a remarkable difference between the flow curves obtained in the saline and the colloid infusion experiments. When the common duct pressure was raised by saline infusion, hepatic arterial flow increased abruptly, reaching a maximum value in about 30 seconds, and remained in most cases at this higher level during the 5 to 15 minutes observation time. In some experiments the flow decreased again gradually but remained above the control value. When the common duct cannula and the reservoir were disconnected, *i.e.* biliary pressure had dropped to zero, hepatic arterial flow returned slowly to the control value. This process took at least 5 to 10, in some experiments considerably more minutes. When destran or serum were infused into the common duct, hepatic arterial flow increased again abruptly, reaching a maximum in 1/2 to 1 minute, but subsequently at sustained high duct pressure, it decreased again rapidly, attaining the control value in less than 3 minutes.

Influence of innervation

The effect of alpha receptor blocking agents was examined in 8 experiments. After a preliminary testing of the hepatic arterial blood flow reaction

to 40 mm Hg common duct pressure, 5 animals received by intravenous injection 0.2—0.4 mg/kg phentolamine. The reaction was tested again 5 to 10 minutes after drug administration. The effect of phenoxybenzamine (2 mg/kg) was studied in 3 animals. The vascular reaction was re-examined 30 to 60 minutes after administration of the blocking agent. Both drugs produced in some cases a small decrease of systemic arterial pressure with nearly unchanged hepatic arterial flow (—6%, $p > 10\%$). The reaction to the increased common duct pressure was not abolished (Table III).

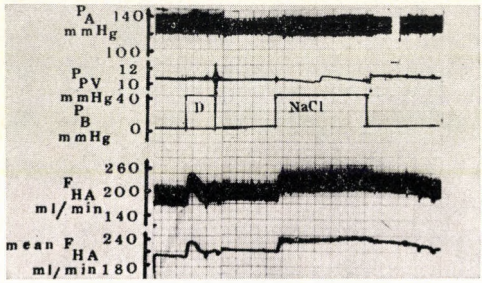

Fig. 5. Arterial flow reactions during the infusion into the common duct of 6% dextran (*D*) and physiological saline solutions (NaCl)

Table III

Influence of innervation on the vascular reaction to an increased common duct pressure

		Control			Examination		
		0 pressure	40 mm Hg	$\Delta F_{HA}\%$	0 pressure	40 mm Hg	$\Delta F_{HA}\%$
		F_{HA} ml/min			F_{HA} ml/min		
Alpha blockade							
N = 8*	$\bar{X}$	296	402	35.5	282	381	35.1
	S ±	18	31	6.7	23	34	7.2
Beta blockade							
N = 8**	$\bar{X}$	270	356	33.4	272	362	33.5
	S ±	34	48	8.3	34	51	5.8
Atropine							
N = 7	$\bar{X}$	272	351	30.8	290	378	30.8
	S ±	31	38	5.7	28	35	7.8

* 3 animals received phenoxybenzamine, and 5, phentolamine
** 3 animals received propranolol, and 5, Trasicor®

The effect of beta blocking agents was also examined in 8 animals. Three animals received by the venous route 0.2 to 0.3 mg/kg propranolone, and 5 animals 0.3 to 0.4 mg/kg Trasicor®. Testing of the response to the raised com-

mon duct pressure was repeated 3 to 5 minutes after drug administration. The drugs had practically no effect of systemic blood pressure and hepatic blood flow and the reaction to increased common duct pressure was repeatedly raised, an unchanged blood flow response was obtained.

The effect of atropin was studied in 7 dogs. The animals received after the preliminary examination of the effect of increased bile duct pressure 0.5 to 1.0 mg/kg atropin by the venous route. The manipulation of raising the bile duct pressure was repeated 5 to 10 minutes after drug administration, and an unchanged blood flow response was observed.

Finally, the vasodilatory response could be elicited even during vasoconstriction induced by norepinephrine. In 3 animals the raised common duct pressure increased hepatic arterial flow by 57%. During the infusion of 10 μg/min of l-norepinephrine, the same intervention produced a 47% increase.

Discussion

Occlusion of the common duct leads to a characteristic reaction in liver circulation. Hepatic artery flow rises when biliary pressure is increased and this may or may not be followed by a decrease in potal venous flow. The arterial reaction has the following characteristics. *a)* When bile pressure is raised gradually at first arterial flow does not change, or increases just slightly. At a critical pressure of about 30 mm Hg, the flow increases suddenly. When biliary pressure is raised above this value, little or no further change occurs in the flow. *b)* A sudden increase of biliary pressure above the critical level leads to an immediate maximum flow response. The response is identical in the same animal at repeated stimulations. *c)* The reaction does not seem to be elicited by the stimulation of some stretch receptor situated in the wall of the common duct. This is supported by the observation that the composition of the fluid influences the course of the reaction. *d)* The reaction does not depend on neurogenic impulses since the blockade of alpha and beta receptors and of the parasympathetic nerve endings does not influence its intensity.

The above characteristic features of the vascular reaction may be explained by a myogenic relaxation of the arteriolar wall due to the decrease in transmural pressure subsequent to the increase of perivascular pressure. Interstitial pressure is increased by the extravasation of bile (and of infusion fluid). It seems, however, that the transmural pressure figuring in this reaction is not determined by the arteriolar and interstitial hydrostatic pressures alone, but rather by the gradient between effective arteriolar and interstitial pressures *i.e.* by the relationship

$$P_t = (P_v - \pi_p) - (P_i - \pi_i)$$

where P_t = transmural pressure; P_v = vascular hydrostatic pressure; π_p = = plasma colloid osmotic pressure; P_i = interstitial hydrostatic pressure; and π_i = colloid osmotic pressure of the interstitial fluid. This conclusion was based on the action of colloid solutions. When the pressure in the biliary ducts is raised by dextran or plasma infusion, first the bile inside the bile ductules and the bile canaliculi is pressed out into the interstitium. This increases interstitial hydrostatic pressure and produces a typical vascular reaction. Later, when the infusion fluid has reached the small bile channels, the interstitium

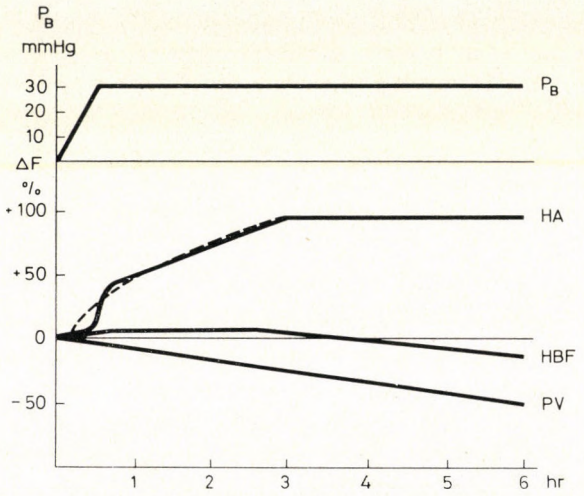

Fig. 6. Changes in hepatic circulatory parameters during an acute increase of biliary pressure. (Composite graph uniting the results of the two types of experiment.)

is flooded with colloid solution, consequently its colloid osmotic pressure rises, the effective arteriolar transmural pressure increases again, and the myogenic vascular reaction is abolished.

Comparing the present results with the data obtained in previous experiments, a remarkable difference was detected. In the present experiments, after raising the biliary pressure above the critical level, an immediate and apparently maximum increase of flow of about 35—40% occurred. In more protracted experiments [8], after occlusion of the common duct, biliary pressure attained its maximum in about 1/2 to 1 hour. The increase in hepatic artery blood flow continued, however, up to the end of the second or third hour, reaching a maximum increase of about 90%. After this, hepatic arterial flow remained constant during the entire 6 hr observation time. When the data from the two types of experiment are combined (Fig. 6), it is seen that the observations are consistent with a two-step arteriolar vasodilatation. This behaviour can easily be explained by the structure of the hepatic arterial bed

[2, 3]. The branches of the hepatic artery accompany in the portal tracts the biliary ducts and the branches of the portal vein. Fine branches of the hepatic artery supply a dense capillary network, the peribiliary plexus, in the connective tissue of the portal canal. Other intralobular branches, after leaving the portal tract join directly the sinusoids. When biliary pressure is increased, the fluid escaping from the paraportal ductules raises rapidly the interstitial pressure in the confined space of the portal canal. By this an arteriolar dilatation is triggered in the peribiliary plexus. With sustained bile stasis, fluid slowly accumulates in the liver parenchyma. This leads to a progressive relaxation of the intralobular arterioles, until finally these vessels are also dilated to the maximum. It can be calculated that the rapidly reacting (peribiliary?) arterioles represent about 35% of the total number or cross section of the hepatic arterioles.

In the present experiments, flow changes in the portal vein were small (−9.5%) and inconsistent. No correlation was found between the alterations of arterial and venous flow. Data from the previous investigations show that the decrease of portal flow is progressive during the entire time of observation. In the first hour, portal vein flow decreased by 17%, and by the end of the 6th hour a 50% decrease is attained. This can be explained by a progressive fluid accumulation in the liver parenchyma and increased sinusoidal and portal inflow resistance. It was pointed out previously that the main cause of the decrease in portal flow is not the increase of portal resistance itself, but a myogenic arteriolar vasoconstriction in the prehepatic splanchnic vascular bed [8].

Statistical analysis was strongly suggestive of a relationship between the changes in portal venous and hepatic arterial blood flows. The interaction of the two vascular systems in the liver is a much disputed question. The observations showed that a decrease of portal pressure or flow consistently leads to an increase in hepatic artery flow [5, 7, 9]. The changes in hepatic artery flow or pressure seem to produce only inconsistent effects on the portal circulation [1, 4, 5, 6, 9]. In the present experiments it was obvious that there is no interaction between the early, immediate arterial reaction and the observed minor decreases of portal venous flow. For the second phase of the arterial reaction, beside the explanation given in the previous paragraphs, an alternative hypothesis could be envisaged. Accordingly the prolonged and continuous increase of hepatic artery flow may be due to the decrease of portal flow. The exact mechanism, by which a change in portal flow leads to arteriolar dilatation is not clear. The most probable explanation would again be myogenic reaction triggered by the changes in sinusoidal haemodynamics.

REFERENCES

1. BOLLMANN, J. L., KHATTAB, M., THORS, M., GRINDLAY, R. & J. H.: Experimentally produced alterations of hepatic blood flow. Arch. Surg. **66,** 562—569 (1953).
2. BRAUER, R. W.: Autoregulation of blood flow in the liver. Circulat. Res. **1,** 213—221 (1964).
3. ELIAS, H.: Functional morphology of the liver. Res. Serv. Med. **37,** 26—51 (1953).
4. GINSBURG, M., GRAYSON, G.: Factors controlling liver blood flow in the rat. J. Physiol. (Lond.) **123,** 574—602 (1954).
5. HANSON, K. M., JOHNSON, P. C.: Local control of hepatic arterial and portal venous flow in the dog. Amer. J. Physiol. **221,** 712—720 (1966).
6. KOCK, N. G., HAHNLOSER, P., RODING, B., SCHENK, W. G.: Interaction between portal venous and hepatic arterial blood flow. An experimental study in the dog. Surgery **72,** 414—419 (1972).
7. SCHENK, W. G. jr., MCDONALD, J. C., MCDONALD, K., DRAPANAS, T.: Direct measurement of hepatic blood flow in surgical patients: With related observations on hepatic flow dynamics in experimental animals. Ann. Surg. **156,** 463—471 (1962).
8. SZABÓ, G., JAKAB, F., MAGYAR, Z.: The effect of acute cholestasis on liver circulation. Acta Med. Acad. Sci. Hung. **31,** 231—241 (1975)
9. TAKEUCHI, J., KUBO, T., TONE, T., TAKADA, A., KITAGAWA, T., YOSHIDA, H.: Autoregulation and interaction between two vascular systems in dog liver. J. appl. Physiol. **27,** 77—82 (1969).

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Acta Medica Academiae Scientiarum Hungaricae, Tomus 31 (3—4), pp 251—255 (1974)

COMPLICATIONS OF POLYCYTHAEMIA VERA AND ITS ASSOCIATION WITH NON-HAEMATOLOGICAL DISEASES

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Received October 29, 1974

The vascular complications of polycythaemia vera and its association with other diseases is discussed on the basis of 156 cases observed over 14 years. Of the patients, 48 had had vascular complications of some kind before the start of active treatment. The frequent occurrence of hypertensive disease, congestive heart failure, angina pectoris, gastric ulcer and of diabetes including diabetoid blood-sugar tolerance curves in patients with polycythaemia vera has been borne out by the present observations.

Polycythaemia vera (PV) is amenable to therapeutic measures and takes a protracted course in case of successful treatment. By contrast, inadequate management in consequence of an erroneous diagnosis involved irreversible or fatal, mostly vascular, complications [7, 12, 13, 18]. On the other hand, suitable treatment with regular haematological follow-up results in longer survival and ensures long-term rehabilitation of those who are still in active age [5, 7, 14, 15, 21].

While the longer survival has allowed to study the pathological and clinical features of the process at a closer range, early diagnosis and management of the associated diseases have become important. These considerations have prompted us to study the vascular complications and the types and incidence of associated diseases in patients observed in the period 1959 to 1972.

Material and method

A total of 156 patients with PV were treated and followed up between March, 1959, to December, 1973. Their average age was 54.1 years, the lowest age-limit was 17 years. Twelve patients were between 20 and 30 years of age. The male-to-female ratio was 1.6 : 1.

All the patients had repeated courses of ^{32}P and/or of cytostatic treatment which resulted in lasting remission in 90 to 95%. The response to repeated treatment made necessary by recurrences was practically the same as on the first occasion [12, 14, 15].

The criteria of diagnosis, the therapeutic schemes (^{32}P, cytostatics) the responses obtained, as also the critera and duration of remissions, have been detailed earlier [11, 13, 14].

In addition to routine tests (erythrocyte, leucocyte, platelet counts, haemoglobin, haematocrit, reticulocyte count, differential count, GAP, bone marrow catology, serum iron, total iron-binding capacity, etc.), liver and kidney functions, and blood sugar tolerance curves were studied in every case. Additional investigations such as respiratory studies, blood gas analysis, gastric radiography, urography, aortography or other examinations were performed when needed. Although dyspnoea of every origin may occur in patients with PB, the difficulties of differentiation from secondary polycythaemia required the exclusion of all cases from

the study in which this possibility could not be ruled out. As regards the complications, the patients were regarded as hypertensive if systolic pressure persisted over 160 mm Hg and diastolic pressure over 90 mm Hg. Thrombosis or thromboembolic episodes were qualified as vascular complications as also those manifestations which were in a causal connexion with the rheological and haemodynamic disturbances related to the primary disease. In contrast, all conditions were qualified as "associated", which, though being more frequent in PV patients than in the age-matched general population, bear no certain relationship with the primary disease. For obvious reasons, the "accompanying" conditions showed a fairly close relationship with the duration and stage of PV, while in the case of "associated" conditions these relationships were less definite.

Results

The vascular complications were divided into four groups, *a)* cerebrovascular crises associated with parolysis; *b)* extremital thrombosis associated with embolism; *c)* myocardial infarction; *d)* haemorrhages (Fig. 1). Vascular

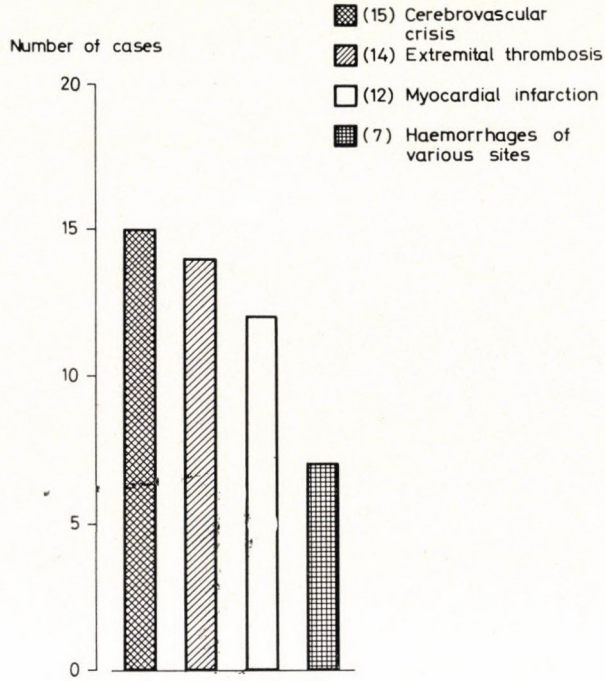

Fig. 1. Common acute complications of PV

complications of some kind occurred in 48 of the 156 cases (31%), before admission *i.e.* the start of active therapy. They included cerebrovascular crises in 15 cases (10%) and acute myocardial infarction in 12 cases (8%). In a number of patients these conditions had directed attention to the possibility of PV. On the other hand, in those patients who had adequate active treatment and

regular haematological follow-up, the incidence of vascular complications was only 2% (3 cases).

The "accompanying" conditions included, in addition to the acute vascular complications, hypertensive disease in 63 cases (40%), congestive heart failure in 60 cases (39%), angina pectoris in 58 cases (37%) and peripheral arterial disease in 17 cases (11%) (Fig. 2). Their incidence began to rise after

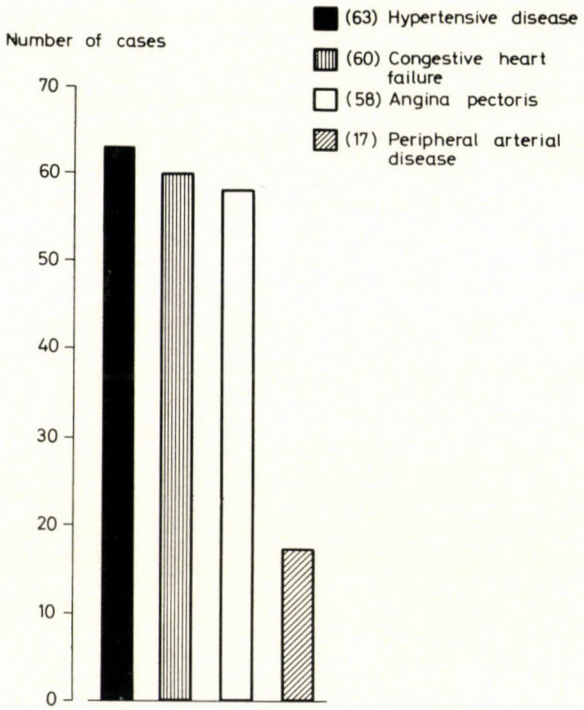

Fig. 2. Incidence of chronic accompanying vascular diseases in PV

a history of PV of 3 to 4 years, and they were practically always present, though of variable severity, after a duration of 7 to 8 years. The observation that the remissions of PV went hand in hand with an improvement of the complications seems to confirm their relation to the primary disease.

The incidence of "associated" diseases is shown in Fig. 3. The most frequent "associated" conditions were diabetes in 35 cases (23%) and peptic ulcer in 28 cases (18%). It was, however, only in the case of peptic ulcer that a parallelism between its clinical course and that of PV was observed; the exacerbations coincided in most patients with the recurrences of PV. Chronic hepatitis was found in 17 cases (11%), chronic renal disease with diffuse parenchymal lesion (chronic nephritis, chronic pyelonephritis, chronic interstitial

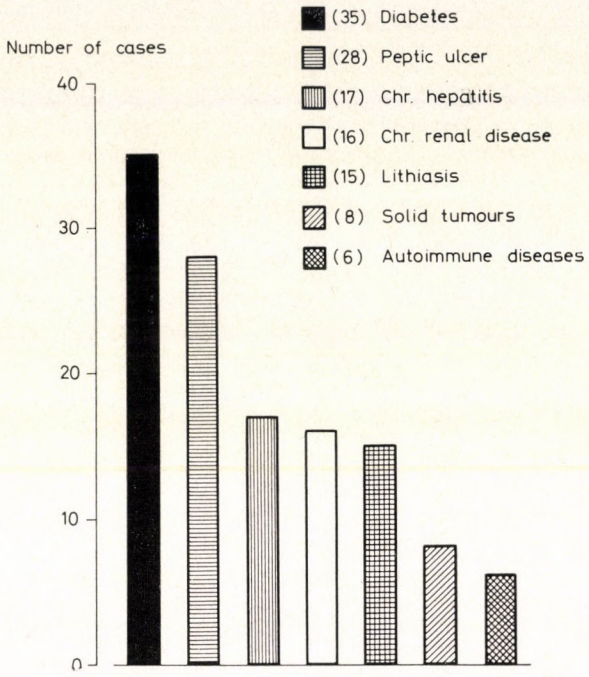

Fig. 3. Common association of PV with other diseases

nephritis) in 16 cases (10%). These two conditions were frequent in patients with a history of PV of 6 to 8 years or longer. Nephrolithiasis and cholelithiasis occurred in 15 cases (10%).

Discussion

In chronic diseases which are amenable to therapeutic measures, survival and working capacity of the patient greatly depend on secondary diseases. LAWRENCE [7], in a long-term follow-up of 231 PV-patients found an association between PV and hypertensive disease in 50%. Of our 156 patients, 63 (40%) were hypertensive. According to DOERING and WENKER [4] and to MALIZA [9] the renal pressor mechanisms stimulated by the renovascular lesions are closely involved in the pathogenesis of hypertensive disease. LOGINOV [8] believes that nervous mechanisms also had a part in eliciting the hypertensive disease accompanying PV.

LAWRENCE [7] found a high incidence of congestive heart failure (34%) and of angina pectoris (16%) in PV.

ROSENTHAL and BASSEN [16] and TINNEY et al. [20] estimated the incidence of peptic ulcer in PV at 10 to 20%. They attribute the high susceptibility to peptic ulcer in PV to the decreased velocity of blood flow and to thrombosis of the small vessels of the gastric mucosa.

The cause of the high incidence of chronic hepatitis in PV is unclear. On the evidence of microscopic studies, SOHVAL [17] found fatty degeneration, MOSSE [10] and LOGINOV [8] accumulation of destruction products of erythrocytes and consecutive fibrosis of the liver.

In the literature available we could find no indication of any close association of PV with diabetes. Yet, in 15 of the present 158 cases we either found a diabetoid blood sugar tolerance curve or, in 20 patients, manifest diabetes requiring treatment and a dietary regimen.

Some renal diseases, in the first place certain renal tumours, are associated with secondary polycythaemia [1, 6, 19]. DELAMORE et al. observed renal dislocation and rotation in 21 out of 25 PV-patients [2]. In the literature there is no evidence of a frequent association-rate of PV with chronic glomerulonephritis or chronic pyelonephritis.

REFERENCES

1. BRANDLEY, J. E., YOUNG, J. D., LENTZ, G.: Polycythaemia secondary to phaeochromocytoma. J. Urol. (Baltimore) **86,** 1 (1961).
2. DELAMORE, J. W., MACDONALD, A. F., SAMUEL, E.: The radiological investigation of polycythaemia with particular emphasis on the renal tract. Brit. J. Radiol. **35,** 671 (1962).
3. DEMIDOVA, A. V., DANYLITSHEVA, Z. M.: Probl. Gematol. **8,** 56 (1966).
4. DOERING, F., WENKER, H.: Nierenfunktion und intrarenale Hämodynamik bei der Polycythaemia rubra vera. Klin. Wschr. **38,** 1028 (1956).
5. HARMAN, I. B., LEDLIE, E. M.: Survival of polycythaemia vera patients treated with radioactive phosphorus. Brit. med J. **2,** 146 (1967).
6. IVÁNYI, J., HEIM, V.: Polycythaemia vera és phaeochromocytoma együttes előfordulása. Orv. Hetil. **104,** 2050 (1963).
7. LAWRENCE, J. H.: Polycythaemia: Physiology. Diagnosis and Treatment Based on 303 Cases. Gruene and Stratton, New York, 1955.
8. LOGINOV, A. S.: C. Sc. Thesis, Moscow 1953.
9. MALIZA, E.: Renal function and hemodynamics in primary and secondary polycythaemia. Acta med. scand. **154,** 399 (1956).
10. MOSSE, M.: Über Polycythämie mit Urobilinikterus und Milztumor. Dtsch. med. Wschr. **52,** 2175 (1907).
11. NAGY, GY.: Diagnosztikus és therápiás problémák polycythaemia vera és primer tüdőtumor tágulásánál. Magy. belorv. Arch. **19,** 272 (1966).
12. NAGY, GY.: C. Sc. Thesis, Debrecen 1968.
13. NAGY, GY.: Polyglobuliák. Orv. Hetil. **111,** 1743 (1970).
14. NAGY, GY.: Polycythaemia rubra vera. Klinikai kép, pathológia és therápia. Az orvostudomány aktuális problémái. Medicina, Budapest 1971/2.
15. NAGY, GY.: A polycythaemia rubra vera (PRV) diagnosztikai és gondozási problémái a körzeti orvosi gyakorlatban. Med. Univ. In press.
16. ROSENTHAL, N., BASSEN, F. A.: Course of polycythaemia. Arch. intern. Med. **62,** 903 (1938).
17. SOHVAL, A.: Hepatic complications in polycythaemia vera. Arch. intern. Med. **62,** 925 (1938).
18. SZUR, L., LEWIS, S. M., PATH, M. C.: The haematological complications of polycythaemia vera and treatment with radioactive phosphorus. Brit. J. Radiol. **39,** 122 (1966).
18. THURMAN, W. G., CRABSTALD, H., LIEBERMAN, P. H.: Elevation of erythropoetin levels in association with Wilms Tumor. Arch. intern. Med. **117,** 280 (1966).
20. TINNEY, W. S., HALL, B. E., CIFFIN, H. L.: Polycythaemia vera and peptic ulcer. Proc. Mayo Clin. **18,** 27 (1943).
21. VARELA, J. E., ROCHNA, V. E. M., CARMENA, A. O., ETCHEVERRY, M. A., KREMENCHUZKY, S.: Polycythaemia vera. Results of repeated radioisotope studies in 53 patients during five year period. Nucl. Med. **3,** 1 (1962).

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RECENSIONES

F. RÉNYI-VÁMOS, A. BABICS: *Anuria. Therapeutic Experiences.* Publishing House of the Hungarian Academy of Sciences, Budapest 1972. 199 pages.

The subject is introduced by a historical review and terminological definitions, then the theoretical and practical problems of acute anuria are discussed. The book contains six chapters. In the first the disorders of fluid and electrolyte metabolism and their various clinical aspects are outlined.

The second and third chapters deal with then pathological, pathophysiological and pathogenetic issues. This part deserves particular attention; it is integrating the results of animal studies with experience derived from clinical observations.

The fourth chapter discusses the clinical aspects of anuria, the clinical features of its successive phases and the consecutive biochemical abnormalities. Questions of prognosis and of lethality are presented in the light of the authors' personal observations confronted with published evidence.

The fifth chapter gives a comprehensive review of the management of acute anuria, including the chances of therapy. The tasks connected with the treatment of the initial lesion and with the subsequent phases of anuria are described in detail. The indications and contraindications of extrarenal detoxification, the possibilities of haemodialysis, peritoneal dialysis, intestinal dialysis and of exchange transfusion are also discussed.

In chapter six the patient material treated for acute anuria at the Artificial Kidney Unit, Department of Urology, Semmeweis University Medical School, Budapest, is reviewed the cases being grouped on the basis of aetiology. The observations summed up here offer valuable practical information.

This book is actually a clinical study. The personal observations, some of which are new and highly relevant, have added to the data published in the literature and represented here in a list of 247 references. All statements express a well-balanced, uniform attitude. The fact that the discussions of the various clinical situations are mirorring the authors' personal observations, greatly enhance the book's value. Clear arrangement, concise presentation of the individual subjects, in particular of the disorders of fluid- and electrolyte metabolism as well as of the pathogenetic problems, give the book a structural consistency which helps the student to see the widely involved aspects.

In dealing with the diagnostic questions posed by acute anuria, the authors stress the advantages of surgical biopsy requiring exposure of the kidney over those of needle biopsy. Attention must be drawn to the observation that renal biopsy of the reveals microscopic signs of an earlier lesion which would seem to suggest that preexisting damage to the kidney might play a part in the production of shock-kidney.

The diagnostic importance of instrumental urological examinations in acute anuria is emphasized and convincingly illustrated by individual observations.

The primary importance of competent, experienced handling is underlined by deterrent examples to the contrary.

It is a particular merit of the book that the possible complications are described with an exemplary objectivity and openness. This will be highly valued by the student of the question since he must be aware of the complications he has to face.

The case reports are instructive and should help to avoid the pitfalls involved at the management of acute anuria.

In sum, the book outlines the relevant facts of pathophysiology, pathology and pathogenesis, it presents observations of an extensive patient material in a clearly arranged form, and indicates the basic lines of conduct in the management of acute anuria on the basis of these observations. It offers, therefore, valuable aid in the university studies of the undergraduate as well as in postgraduate training.

J. PINTÉR

Autoimmun betegségek. (Autoimmune diseases) Editor: GY. PETRÁNYI. Akadémiai Kiadó, Budapest 1974. 434 pages.

This book contains the contributions of 16 authors who have been engaged in the study of the various pathogenetic, diagnostic and therapeutic aspects of autoimmune diseases for many years. The editor has written most of the chapters. There are three main sections; I. general aspects; II. the autoimmune syndromes; and III. laboratory diagnostics.

I. The general section gives a clear, concise outline of the basic facts concerning the autoimmune syndromes. The recent advances in this field receive thorough consideration. The discussions are centred on the individual types of immune reaction and on the various theories of pathogenesis.

II. The second part covers the individual autoimmune syndromes. Gy. Petrányi gives an exhaustive review of systemic lupus erythematosus presenting its various aspects in the light of personal observations. Then the monosystemic, organ-specific syndromes are discussed. Scleropathy, dermatomyositis, pemphigus and the Moschcowitz-syndrome are dealt with by L. Szodoray. The contribution by K. Király represents a novel approach to the autoimmune implications of the various types of vasculitis which still form a highly controversial issue. Numerous excellent illustrations add informative value to the descriptions. In the chapter on the immunohaematological involvement of autoimmunity there is a contribution by Zs. Hollán on the autoimmune disturbances erythropoiesis, and by E. Kelemen on those of the leukopoietic and thrombopoietic systems. The latter author gives due emphasis to the difficulties in ascertaining the autoimmune origin of thrombocytopenia or of granulocytopenia in a given case, owing to the uncertainty of demonstration of specific antibodies. The relevant facts of immunoendocrinology are presented by Gy. Petrányi, particularly as concerns autoimmune thyroiditis and Graves' disease. The autoimmune diseases of the serous membranes, lung, heart, large vessels, kidney and digestive organs are discussed by the same author. S. Dán describes the autoimmune diseases of the liver, in particular their well-known forms but gives no sharp definition of the laboratory and pathohistological diagnostic criteria of the individual syndromes. Section II further includes the autoimmune diseases of joints, bones, muscles, connective tissue, nervous system (A. Szobor) and of the eye (B. Albert). A chapter is devoted to the relationships of the autoimmune syndromes and immune deficiency (L. Schöngut and I. Rényi), a further chapter deals with the connections between these syndromes and malignant disease (Gy. Szegedi). It is convincingly shown in both chapters that the prevalence of the two last-named conditions increases in proportion to the duration of primary immune

deficiency. The autoimmune and malignant syndromes associated with immune deficiency might show some light on the pathomechanism of these conditions.

Section III is devoted to laboratory diagnostics. R. Backhausz deals with the structure of immunoglobulins, the relationships between antibodies and immunoglobulins, the properties and classification of paraproteinaemias, then the immunochemical procedures for the demonstration of immunoglobulins and paraproteins are described.

In the closing chapter we find a clear description of the various procedures for the study of humoral and cellular immunity (J. Bobory) and of those of immunochemistry (E. Berger), illustrated by numerous instructive graphs.

A. PATAKFALVI

L. HARANGHY, K. SZEMENYEI: *Pathology of Tuberculosis in Old Age.* Publishing House of the Hungarian Academy of Sciences, Budapest. 210 pages with 66 illustrations.

In the possession of efficient weapons against tuberculosis there is a general tendency to regard the fight against this disease, at least in the developed countries, as imposing merely organizational tasks. Convincing evidence against this belief is furnished by this book in the form of post-mortem evidence obtained in 200 subjects who had contracted tuberculosis beyond the 5th decade. Analysis of this material reveals that grave forms of tuberculosis, often of fatal outcome, are by no means uncommon in the aged even in our days; that these tuberculous changes are generally of utmost severity and tend to assume exudative-caseous features; that their clinical course is often atypical. It is due to the atypical clinical features in the elderly on the one hand, and to the prevailing tendency on the other, to dismiss the possibility of tuberculosis as being non-existent in our days, that the process remains undetected in many cases, the more so as in this period of life tuberculosis is often masked by other diseases.

The subjects discussed in the framework of 9 chapters include the incidence of tuberculosis in the aged, its clinical aspects, its pathological features, the extrapulmonary forms affecting the lymph nodes, the spleen, the bone marrow and the kidney, other diseases associated with tuberculosis in the elderly, and the various factors affecting tuberculosis in the aged.

The text is made still more convincing by many illustrations and microphotographs. An exhaustive bibliography is a further asset of the book.

The interest of the monograph is by no means confined to physicians engaged in the fields of tuberculosis or of geriatrics. It will be valued by all those desirous to keep abreast of the advances in medicine. In fact, the book fills a long-felt gap.

I. FÖLDES

Symposium on gastrin and its antagonists (Vol. 3. of the Proceedings of the First Congress of the Hungarian Pharmacological Society, Budapest 1971.) General editor, J. KNOLL; Editors J. BORSY and GY. MÓZSIK. Publishing House of the Hungarian Academy of Sciences, Budapest 1973. 153 pages.

The volume comprises the papers delivered at a section of the First Congress of the Hungarian Pharmacological Society, held with the participation of many internationally known experts. Although the full text of the papers has been published with a delay of two years, they are still timely. It is still unclear which physiological mechanism, more precisely which substance, inhibits the effect of gastrin, the hormone stimulating hydrochloric acid production during the autoregulation of gastric hydrochloric acid secretion. Accordingly, it is still a most timely and at the same time challenging task for pharmacologists to synthesize some antigastric compound that would exert a therapeutic effect on gastric hypersecretion or peptic ulcer. The importance of further investigations in this field is underlined by the failure

of reducing gastric hydrochloric acid secretion through the inhibition of the regulating nerve by anticholinergic drugs, due mainly to their undesirable side effects.

Gastrin is a polypeptide hormone consisting of 17 amino acids. It is isolated from the mucous membrane of the stomach. Its synthetic analogue, pentagastrin, contains only 5 amino acids. Gastrin stimulates hydrochloric acid secretion by inducing the histidine-decarboxylase; thereby it causes histamine accumulation in the gastric mucosa. Histamine participates in the secretary response of the stomach *via* cyclic AMP. Histamine and pentagastrin, as immunosuppressive agents, inhibit the transport ATP-ase (ATP—ADP) system isolated from the gastric mucous membrane. Gastrin also stimulates the peristaltic movements of the small intestine. Besides its other gastrointestinal achives, the caerulein isolated from the skin of the frog *Hyla caerulea* exerts similar effects.

Under physiological conditions, the hydrochloric acid secretion stimulating effect of gastrin is inhibited by bulbogastrone, the polypeptide produced in the duodenal bulb upon the effect of hydrochloric acid. 2-pyridyl-thioacetamide and 3,4,6,7-tetraethoxy-1-benzyl 1,2,3,4-tetrahydroisoquinoline (BFT) inhibit enhanced hydrochloric acid secretion and the development of experimental gastric ulcer by their antigastria effect and not by some anticholinergic mechanism. On the other hand, in clinical practice, BFT failed to prove more effective than a combination of traditional anticholinergic and spasmolytic agents. Proglumine CR 242, also an antigastria substance, accumulates in the gastric mucous membrane and has trophic, antisecretory and antispastic effects.

It is regrettable that the volume fails to give information on the effect of antigastria compounds on gastrin release and concentration.

The illustrations are clear and demonstrative. The volume will be of use to all those engaged in investigating the regulation of gastric hydrochloric acid production.

M. PAPP

Arterial lesions and arteriosclerosis. Edited by H. JELLINEK. Publishing House of the Hungarian Academy of Sciences, Budapest 1974. 331 pages with 433 illustrations.

Research into the pathogenesis of arteriosclerosis has been expanding considerably in the past 15 years. This book gives a good account of experimental investigations into the vascular lesions and model arteriosclerotic changes carried out in the Editor's institute in the past decade.

The book is divided into eleven chapters written by nine authors. It is introduced by a short review of the structure and cellular elements of the mammalian arterial wall. In the following chapter the aortic response to various injuries is discussed with special regard to the submicroscopic structure of the elastic fibres of the aorta and the smooth muscle nature of the cellular elements of intimal proliferation and the role of these cells in the production of elastic fibres. The next chapter deals with the development and ultrastructure of arteriosclerotic lesions. In the following four chapters, various aspects of experimental vascular lesions are dealt with: acute vascular lesions induced by experimental hypertension, the formation and ultrastructure of subendothelial fibrinoid and the histochemistry of acute vascular lesion followed by the morphological demonstration of their common outcome, the chronic proliferative intimal lesions with hyaline degeneration of the arterial wall. An interesting chapter is devoted to the ultrastructural phenomena of the transport process across the vascular wall, and the conclusion is reached that the pathologically altered transport process of plasma substances through the vessel wall plays the primary role in the development of vascular changes including arteriosclerosis. Changes in human vascular diseases and arteriosclerosis are compared morphologically with similar changes induced experimentally in animals. An appendix de-

scribes the most important methods used. 245 excellent black-and-white, mostly electron microscopic pictures and 188 colour figures accompany the book.

The monograph offers a wealth of information on the recent progress of vascular pathology with a unifying attitude concerning the development of arterial lesions. Its basic idea is that "the tissue characteristics of vascular changes depend on the degree and duration of the injury and on the structure of the vessel wall, but do not depend on the nature of the injury". Supplemented with the most recent data in the literature (a comprehensive bibliography of 40 pages) the book will be of value for all those who are interested in the pathomechanism of vascular lesions in general and in arteriosclerosis in particular.

G. Romhányi

25th CONGRESS OF THE HUNGARIAN SOCIETY FOR CLINICAL PATHOLOGY

PÉCS 29-31. AUGUST 1974

ABSTRACTS

INDEX

RENIN, ANGIOTENSIN UND ALDOSTERON IN DER KLINISCHEN DIAGNOSTIK

H. P. WOLFF

I. MEDIZINISCHE UNIVERSITÄTSKLINIK, MAINZ, BUNDESREPUBLIK DEUTSCHLAND

Abkürzungen: PRC = Plasmareninkonzentration
ASR = Aldosteronsekretionsrate
PAC = Plasmaaldosteronkonzentration

Bestimmungen von PRC u./o. ASR o. PAC sind zweckmäßig bzw. notwendig:

1. Bei Verdacht auf renovaskuläre Genese eines Hochdrucks (Nierenarterienstenose, »kleine« Niere entzündlicher, vaskulärer, oder hypoplastischer Genese). Erhöhung der PRC im Nierenvenenblut und Abnahme der Plasmadurchströmung (^{131}J-Hippuran-Clearance) der befallenen Niere werden als Beurteilungskriterien der Hochdruckgenese und der Indikation zur chirurgischen Therapie (Revaskularisation bzw. Nephrektomie) betrachtet.

2. Zur Differentialdiagnose der hypokaliämischen Hypertonien: 1. Hypertonie mit sekundärem Aldosteronismus (bei fortgeschrittener oder maligner Hypertonie, renovaskulärer Hypertonie, »kaliumverlierenden« polyurischen Nephropathien sowie bei Langzeittherapie mit Diuretika) = periphere PRC sowie ASR o. PAC erhöht, 2. primärer (M.-Conn) oder pseudoprimärer Aldosteronismus = PRC erniedrigt, ASR o. PAC erhöht. 3. Mineralokortikoidhypertonie ohne Aldosteronismus (11-, 17- o. 18-Hydroxylasemangel = PRC erniedrigt, ASR u. PAC normal DOCSR erhöht. 4. Pseudomineralocorticoidsyndrome.

3. Zur Differentialdiagnose des primären (M.-Conn) und pseudoprimären (Hypertonie mit bilateraler Nebennierenrindenhyperplasie) Aldosteronismus: Gemeinsam ist die Suppression der PRC und die Erhöhung der ASR und PAC. Seitenlokalisation und Darstellung des (r) solitären (r) Adenome (s) durch seitengetrennte Bestimmung der PAC im Nebennierenvenenblut, Nebennieren-Arterio-o.-phlobographie sowie Nebennierenszintigraphie mit ^{131}J-Jod-cholesterol.

4. Zur Abgrenzung der »hyporeninämischen Hypertonie« (PRC erniedrigt, ASR und PAC sowie Gesamtkörperkalium normal) von der unkomplizierten »essentiellen« Hypertonie (PRC und ASR normal) und dem normokaliämischen Stadium eines primären Aldosteronismus PRC (niedrig) normal, ASR (leicht) erhöht und Gesamtkörperkalium (mässig) erniedrigt.

Acta Medica Academiae Scientiarum Hungaricae 31, 1974

ENZYMMUSTER AM NEPHRON

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Mit Hilfe der quantitativen Histochemie, der sogenannten Lowry Technik, gelang es aus Nierengewebeschnitten anatomisch abgrenzbare Abschnitte des Nephrons zu isolieren und an diesen Strukturen Enzymanalysen auszuführen. Die mit dieser Technik erstellten quantitativen Aktivitätshistiogramme von Enzymen vermitteln eine genaue Vorstellung über die metabolische Differenzierung des Nierengewebes. Darüber hinaus geben die Enzymaktivitätsmuster Auskunft über den Funktionstyp dieser Strukturen, nämlich der sogenannten Nephronsegmente.

Eigene Untersuchungen mit Hilfe der quantitativen Histochemie am Nierengewebe der Ratte zeigen quantitative Aktivitätshistiogramme von Enzymen aus Glykolyse, Citratcyklus, Hexosemonophosphatshunt, Aminosäurestoffwechsel und Membrantransport. Aus ihnen geht hervor, daß sich der Aktivitätswert jedes untersuchten Enzymes innerhalb des proximalen und distalen Tubulus ändert. Darüber hinaus lassen beide Nephrontypen signifikante enzymatische Unterschiede erkennen. Diese neuen Befunde zur metabolischen Organisation des Nierengewebes weisen unter anderem auf die Tatsache hin, daß die subtile Kenntnis der zytomorphologischen Differenzierung und der Zytoarchitektonik, sowie die daraus resultierende exakte Identifikation des dissezierten Materials die Grundlage der quantitativen Histochemie am Nierengewebe ist. Die Lowry Technik ist mit der Punktionsmethode der Physiologen vergleichbar; ihre Anwendung bedeutet einen Fortschritt in der Erforschung von Zusammenhängen zwischen Organstruktur und ihrer funktionellen Leistungsbreite.

ENZYMBESTIMMUNGEN IM HARN

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Das Referat enthält:

1. eine kritische Übersicht der bislang hauptsächlich herangezogenen Enzyme,

2. Bemerkungen zur Methodik, zur Beeinflussung von Enzymaktivitäten durch zelluläre Harnbestandteile und zum Problem des Bezugssystems,

3. diagnostische Ergebnisse.

Eigene Untersuchungen beziehen sich vorwiegend auf Lysozym = Muramidase (3.2.1.17), β-Glucuronidase (β-D-Glucuronid-glucuronohydrolase, 3.2.1.31) und Alaninaminopeptidase (L-Alanyl-peptidhydrolase, AAP, 3.4.1.-) samt Isoenzymen. Die verschiedenen Nierenerkrankungen bzw. Funktionsstörungen (glomeruläre, tubuläre Läsion, tubuläre Degeneration) lassen sich durch unterschiedliche Enzymmuster charakterisieren. An weiteren Anwendungen werden besprochen: *a)* Erkennung und Therapiekontrolle der beginnenden Transplantat-Rejektion, *b)* typische Exkretionsmuster nach Injektion von Amidotrisoate-Verbindungen als Funktionstest, *c)* Erkennung von Tubulusschäden bei Cholestase, durch Tuberkulostatika, usw.

NEPHROTIC SYNDROME

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The term nephrosis, first used in 1905 by von Müller and considered to represent a pathological-clinical entity, is to-day a quantitative expression for gross proteinuria, hypoproteinaemia and hypercholesterolaemia.

The classification of the nephrotic syndrome (NS) is based, beside the clinical picture, on chemical and immunochemical methods as well on histology. The chemical and immunochemical methods differentiate

1. Benign proteinuria and proteinuria due to a pathologic process;
2. Glomerular and tubular proteinuria;
3. Secondary and primary, idiopathic, genuine NS.

In the case of idiopathic NS, renal biopsy may reveral

1. Minimal change disease;
2. Focal sclerosis;
3. Membraneous nephropathy;
4. Proliferative
   or mesangial
   or endo-extracapillary
   or membranoproliferative glomerulonephritis;
5. Microcystic renal disease;
6. Diffuse mesangial fibrosis.

THE MECHANISM OF MASSIVE PROTEINURIA IN NEPHROSIS

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In 70 cases of the nephrosis syndrome the urinary proteins were investigated by gel filtration, electrophoresis and immunodiffusion. Gel filtration on Sephadex G200 revealed only albumin and proteins of low molecular weight. Paper electrophoresis showed alpha−1 and beta globulins of high molecular weight. In our ultracentrifugal studies in 1954 we found that urinary proteins in nephrosis consisted chiefly of low molecular weight protein; gamma globulin isolated from urine was also of low molecular weight. Chromatography and immunological methods showed, however, no close correlation between molecular weight and urinary excretion. IgM was never found in the urine, alpha-2 macroglobulin with nearly the same molecular weight was frequently identified and so were gamma-globulin and coeruloplasmin. IgA was always absent.

A semi-quantitative method using urine concentrates revealed the constant presence of albumin, transferrin, alpha-1 glycoprotein, and alpha-1 antitrypsin. IgG, beta-2 glycoprotein and haptoglobin were frequently present at lower concentrations. It seems that urinary proteins up to a molecular weight of 100 000 are excreted in an unchanged form, while proteins with a higher molecular weight undergo during urinary passage a transformation without a change in their electrophoretic mobility and immune-specifity. A strong immuno-precipitation with F_c and F_d antibodies was found in nearly every case of nephrosis, suggesting an enzymatic decomposition of high molecular proteins during renal passage. In summary, the concept of "selective proteinuria" seems to be correct, but its mechanism is not a simple molecular sieving but presupposes an enzymatic transformation of globulin.

THE BACTERIOLOGY OF PYELONEPHRITIS

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Acute and chronic pyelonephritis are of bacterial infections. In most cases some factors stimulate the bacteria in the urine to grow and to invade the tissues of the kidneys. The most important predisposing factors are pregnancy, diabetes, congenital abnormalities of the urinary tract and disturbances of micturition.

In the overwhelming majority of cases, pyelonephritis is caused by members of the family Enterobacteriaceae. *E. coli* generally accounts for more than 80% of uncomplicated cases, whereas Proteus, enterobacter, enterococci and P. aeruginose are more likely to be found in patients who have had a previous infection. The strains of E. coli are divided into a number of serotypes on the basis of their O-antigen, but most of the strains cultivated from the urine of pyelonephritic patients belong to a small proportion of these serotypes. Filterable forms of bacteria (L-forms, protoplasts, mycoplasma) may play an aetiological role.

According to large scale survey, a certain proportion of the population harbours bacteria in the urinary tract without symptoms. Patients with asymptomatic bacteriuria run a great risk of developing acute urinary tract infections and pyelonephritis.

The bacteria causing pyelonephritis and bacteriuria are easy to cultivate, but the diagnosis meets with difficulties. To overcome them, suitable methods and a good organization are necessary. The more important steps of bacteriologic diagnosis are as follows.

1. Collection of urine under comparatively sterile circumstances. The middle-stream urine is acceptable if the patients and nurses are pre-educated.

2. Quick transport of the urine to the laboratory. Cooling is necessary before inoculation.

3. The urine of the pyelonephritic patient must be inoculated onto a suitable medium. The significance of bacteriuria can be proved with quantitative or semi-quantitative counting methods. A count of 100 000 per ml of urine seems to be acceptable for separating significant and non-significant bacteriuria.

4. In screening surveys, the nitrate reduction or TTC test may be recommended. The coincidence of a positive reduction test and cultivation may reach 85—90%. Positive results should be checked by subsequent cultivation of the urine.

5. Microscopic analysis of the sediment (leukocytes, casts, Addis count) may furnish important data but is not sufficient for the diagnosis of pyelonephritis or symptomless bacteriuria.

AUTOMATION AND COMPUTERIZATION IN CLINICAL CHEMISTRY

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Abstract not submitted.

METHODS OF GFR DETERMINATION

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Abstract not submitted.

DIFFERENTIAL DIAGNOSIS IN URAEMIC EMERGENCY

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Abstract not submitted.

LABORDIAGNOSE BEI PYELONEPHRITIS

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Zusammenfassung nicht erhalten.

PLASMA RENIN ACTIVITY AND ACUTE RENAL FAILURE

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Plasma renin activity has been studied by means of Boucher's modified method in 40 patients in various stages of acute renal failure.

1. Plasma renin activity was increased in the anuric stage and decreased in the polyuric and convalescent stages.

2. The highest increase in plasma renin activity was found in patients with poor prognosis or fatal outcome after crush syndrome or dimethyldipyridium poisoning.

3. An increase of plasma renin activity was observed also after haemodialysis.

4. No correlation was found between plasma renin activity and blood pressure, sodium and potassium level in serum and urine during the various stages of acute renal failure.

RADIOIMMUNOLOGISCHE BESTIMMUNG DER PLASMA-RENIN-AKTIVITÄT

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Zusammenfassung nicht erhalten.

RADIOIMMUNOASSAY FOR ALDOSTERONE IN HUMAN PLASMA

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A simple radioimmunoassay for the measurement of aldosterone in human peripheral plasma was elaborated. 10 ml plasma with ^{3}H-aldosterone (5000 dpm 20 pg) was extracted with dichloromethane. After purification, a single paper chromatography (E2B) was carried out. After elution of the aldosterone spot in methanol and filtration through glass-wool, aliquots were taken for the recovery of ^{3}H-aldosterone and radioimmunoassay. Either an antialdosterone serum from rabbits with an antibody titre of 1 : 12 000 or sheep antiserum (batch 088, received as a gift from the National Institutes of Health, Bethesda Md.) with an antibody titre of 1 : 450 000 was used. Samples and standards were incubated at 4 °C for 24 hrs with the diluted antisera. For the separation of bound and free aldosterone, dextrancoated charcoal was used.

Accuracy of the method:

Recovery of added unlabelled aldosterone to plasma pools gave a correlation coefficient of 0.986, with a regression equation of $Y = 1.102x - 76.5$ pg.

Precision of the method:

Replicate analyses of a plasma sample gave a within-assay variation of 6.5%, and between-assay variation of 11.7%.

Sensitivity of the method was estimated from twice the SD of the zero point; the theoretical limit of detection was 14—16 pg.

Specificity of the test depends on the preparation of a sufficiently purified extract for assay and on the properties of the antiserum. The cross-reaction of different steroids with aldosterone antibodies was calculated according to Abraham (1969).

^{3}H-aldosterone recovery added to plasma pools was $71 \pm 4.9\%$ ($n = 20$).

Plasma aldosterone value for normal supine subjects was 8.2 ± 4.6 ng/100 ml, and after 4 hrs in the upright position and after 80 mg furosemide, 26 ± 8.2 ng/100 ml.

DER EINFLUß ENDOGENER UND EXOGENER FAKTOREN AUF DIE ERGEBNISSE VON SERUMKREATININBESTIMMUNGEN

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1970 wurden in einem Screening 2728 und 1973 im Re-screening der gleichen Stichprobe 1523 Kreatininbefunde erstellt und hinsichtlich möglicher Einflußfaktoren statistisch ausgewertet.

Die Werte wiesen einen hochsignifikanten Geschlechtsunterschied von 15—20% in jeder Altersklasse auf und zeigten bei Männern und Frauen einen Anstieg mit zunehmendem Alter.

Im Screening 1970 wurde ein signifikanter Anstieg der Kreatininwerte um die Mittagszeit beobachtet. Durch gesonderte Untersuchungen und statistische Vergleiche konnte wahrscheinlich gemacht werden, daß dieser Anstieg nicht auf die endogene circadiane Rhythmik, sondern auf die körperliche Aktivität der Probanden zurückzuführen ist. Erkrankungen wie Diabetes, Adipositas, Hypertonie usw., die sekundäre Läsionen der Nieren hervorrufen können, bewirken eine statistisch signifikante Erhöhung der Kreatininwerte gegenüber denen der diagnosefreien Probanden. Daraus ergeben sich wichtige Schlußfolgerungen für die klinische Interpretation von Kreatininbefunden sowie für die Erstellung von Referenzbereichen.

LITERATUR

G. Stolze, H. J. Thiele, Hedi Friedmann, H. J. Tredt, Jutta Stolze und Inge Linden: Dtsch. Ges.-Wes. **29**, 193 (1974).

EMPFINDLICHKEIT UND SPEZIFITÄT DER TESTPARAMETER IM NEPHROPATHIESCREENING

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Für das Nephropathiesieb innerhalb des Multiphasenscreening »Sternberg '70« (2908 Probanden) wurde eine umfangreiche Parameterkombination eingesetzt, um die in der Stichprobe vorhandenen Nephropathien möglichst vollständig zu erfassen und durch Kenntnis von Empfindlichkeit und Spezifität der einzelnen Parameter das Testprogramm optimieren zu können.

Die größte Empfindlichkeit wiesen die Parameter Eiweiß im Harn mit 39,7% und Kreatinin im Serum mit 36,3% auf. Dem qualitativen Proteinurienachweis haftet jedoch der Nachteil einer niedrigen Spezifität (82%) an, die durch das Auftreten sogenannter physiologischer Proteinurien, sowie das häufige Vorkommen bei anderen Erkrankungen (z. B. Hypertonie und Prostatahypertrophie) mit jeweils ca. 24% bedingt ist. Bei semiquantitativer Auswertung der Harneiweißausscheidung und Festlegung eines Schwellenwertes (60 mg Eiweiß/100 ml Harn) und/oder mehrzeitiger Durchführung des Tests läßt sich die Spezifität zur Niere beträchtlich verbessern, was für den Nachsorgeaufwand von erheblicher Bedeutung ist.

Die Spezifität des Serumkreatinins ist mit 92.3% wesentlich höher und läßt sich wahrscheinlich weiter verbessern. Seine große Bedeutung als Screeningparameter wird außer durch die relativ günstigen Kennziffern noch dadurch unterstrichen, daß 24 der insgesamt 102 diagnostizierten Nephropathien nur mit diesem Symptom im Screening auffällig wurden.

Aus den Erfahrungen dieses Nephropathiesiebtests, sowie eines 1973 an der gleichen Stichprobe durchgeführten Re-screening wird eine optimierte Parameterkombination vorgeschlagen.

ISOTACHOPHORESIS OF URINARY PROTEINS*

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In the last few years isotachophoresis has found application in several branches of chemistry. In view of the high concentrating power of the method, isotachophoresis or displacement electrophoresis can usefully be applied for

* This work was supported by a grant from the "Onderling Overlegde Akties" (O. O. A. Brussel) and in part by Grant no 1206 of Nationaal Fonds Geneeskundig Wetenschappelijk Oderzoek, Brussel.

separation, identification and detection of proteins in dilute biological samples. The aim of the present study was to apply agarose as a supporting medium in isotachophoresis, to allow a further immunological characterization of the separated and concentrated protein fractions. The simplicity in handling of this carrier makes the method useful for clinical work.

Isotachophoresis is carried out with a LKB constant current power supply in a LKB electrophoretic chamber (LKB 2117 Multiphor). 0.8% purified agarose, A 37 (Ind. Biol. Française) containing the leading ion was layered on clear glass plates (4 cm to 9 cm). As leading electrolyte we used 0.018 M orthophosphoric acid adjusted to pH 5.5 with the addition of Tris. After gelification, a 2—5 cm part of the gel was cut off an replaced by the terminator gel. As terminating electrolyte we used 0.04 M glycine, adding tris to pH 8.7. Ampholines (LKB) in the pH range 6—8 were introduced as spacers. Voltage was increased from 300 to 600 V and to avoid Joule heating the current was maintained at 8 mA. Reliability of the method was checked with albumin transferrin solutions. $2 \cdot 10^{-8}$ g of each protein and 2 μl of ampholine were analyzed. The separated protein bands were identified by immunological analysis, which proved the sensitivity of the method. Normal urine samples contained traces of albumin. Orthostatic albuminuria showed distinct albumin and transferrin lines. Urine samples of nephrotic patients gave eight distinct lines; these were divided into four main and four minor components. The four main fractions were identified as albumin, transferrin, IgG and IgA.

The great advantage of the method is its concentrating power and the simplicity of handling which makes the technique useful for clinical and experimental work.

ERFAHRUNGEN MIT NEUEN SCHNELLTESTS ZUR HARNUNTERSUCHUNG

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Im Laufe der letzten Jahre und Monate wurden mehrere neue Schnelltests zur Harndiagnostik eingeführt. Ein neuer Teststreifen auf Urobilinogen, der nicht mehr auf der Ehrlich-Reaktion, sondern auf einer viel spezifischeren und empfindlichen Diazoreaktion beruht, erlaubt jetzt einen problemlosen Nachweis und eine halbquantitative Schätzung dieses Pigmentes. Von drei neuen Streifentests auf Bilirubin erwies sich einer wegen ungenügender Empfindlichkeit als völlig unbrauchbar. Die beiden anderen sind für klinische Zwecke genügend empfindlich. Besonders interessant ist ein neuer Teststerifen auf Blut im Harn, der mit Sicherheit Hämaturien ab 10 000 Ery/ml nachweist

und dazu noch eine Differenzierung zwischen intakten Erythrozyten und freiem Hämoglobin erlaubt. Auch Myoglobin wird bereits ab 0.05 μg/ml nachgewiesen (Myoglobinurie bei Herzinfarkt). Die Empfindlichkeit eines Teststreifens auf Vanillinmandelsäure liegt leider bei 20 mg/l, was zur Phaeochromozytomdiagnose nicht ausreicht. Die seltenen Neuroblastome scheinen jedoch stets erfaßbar.

Bei allen besprochenen Tests wird auf Wirkungsmechanismus und Störungsmöglichkeiten näher eingegangen.

PROTEINURIE-BESTIMMUNG MIT HILFE VON PAPIERTEST

V. Svoboda

LACHEMA, BRNO, TSCHECHOSLOVAKEI

Man vergleicht einzelne Labormethoden der semiquantitativen Bestimmung der Proteine im Harn mit spezieller Hinsicht zur Verwendung der Papierteste. Es werden die Vorteile des diagnostischen Papiers Albuphan diskutiert.

THE DIAGNOSTIC SIGNIFICANCE OF ISOTOPE RENOGRAPHY

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Within a six year period, some 1200 isotope renograms were performed on clinical patients and outpatients. The renograms were obtained by the intravenous injection of ^{131}I Na-iodohippurate and its detection by scintillography and ratemeter-recorders. In the last three years, the "Radionephrograph" Type MB 7104 of BFKI has been used. The results showed a high efficiency of the method to reveal unsuspected unilateral renal failure mostly with normal blood and urine chemistry.

Unilateral or bilateral ureteral obstruction was also unequivocally indicated even with unaffected tubular function.

The problem of blood supply to the transplanted kidney could also be succesfully investigated.

Diagnosis of unilateral or bilateral renal artery stenosis as a cause of hypertension poses some problems, as quantitatively evaluable data can be masked by differences in size and/or abnormal site of one or both kidneys.

Attempts at the quantitative evaluation of renal function by graphic analysis of individual renograms showed a poor correlation with conventional renal function tests (specific gravity, clearance, urinary N-concentration).

QUANTITATIVE EVALUATION OF THE ISOTOPERENOGRAM OF PYELONEPHRITIC CHILDREN

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Isotoperenography was performed on 32 children suffering from acute and chronic, primary or secondary pyelonephritis. The course of the disease was followed up and compared with other diagnostic criteria.

Renal function was estimated by the mathematical evaluation of the second phase of renograms, because in this phase the kidney accumulates the tracer and the degree of accumulation is in strict correlation with tubular function. The initial value of the second phase, A, was subtracted from the 3rd minute value and divided by the numerical value of $A\left(\frac{3'-A}{A}\right)$. This uptake ratio is independent of the magnitude of the administered dose of the tracer and gives a figure of 0.5—2.1.

The investigations were carried out with a four-channel equipment (Nephrograph NM 7104 made by EFKI Hungary) in the supine position. ^{131}I iodohippurate of 0.5 μCi per kg body weight was given intravenously.

Uptake in the second phase of the renogram as compared to the previous result for the same child revealed a deterioration of renal function in 9 cases (in 3 cases on both sides, in 2 cases on the left side and in 4 cases on the right side).

Among these 9 patients, the progression of pyelonephritis was clinically evident in 6 cases; in 3 cases there was no sign of deterioration.

In 23 cases, renal function did not deteriorate. In 13 cases there was no change and in 10 cases an appreciable improvement was seen.

Out of these 23 cases the infection persisted in 4 cases and became intermittent in 6 cases.

It is concluded that the numerical evaluation of the second phase of the renogram offer more information as to the course of the disease and the effect or failure of treatment than does a semi-quantitative estimation.

EINIGE PROBLEME DER HARNSTOFF- UND KREATININ-BESTIMMUNG

V. CHROMY

LACHEMA, BRNO, TSCHECHOSLOVAKEI

Es werden die Probleme der Harnstoff- und Kreatinin-Bestimmung mit Hilfe von spektrophotometrischen Methoden, Reaktionsmechanismus und Standardisierungsprobleme diskutiert. Man führt optimale Bedingungen für die Harnstoff-Bestimmung durch Diazetylmonoxim und Kreatinin-Bestimmung durch die Jaffe-Reaktion an.

EINE MODIFIZIERTE BESTIMMUNG DES KREATININS IN SERUM UND HARN

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BOEHRINGER MANNHEIM GMBH, MANNHEIM, BUNDESREPUBLIK DEUTSCHLAND

Durch eine Modifikation nach Bernt wurde die Empfindlichkeit des bisherigen Tests erhöht. Der Meßbereich wurde erweitert. Dadurch ist es möglich, zwischen 500 und 550 nm in 1 cm-Küvetten zu messen.

Eine kinetische Durchführung der Kreatinin-Bestimmung erspart die Enteiweißung.

Beide Methoden werden mit dem Verfahren nach Adsorption an Fullererde (Referenz-Methode) verglichen.

A SIMPLE KINETIC, SPECTROPHOTOMETRIC PROCEDURE FOR CREATININE ASSAY IN THE PRESENCE OF PROTEIN

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The only suitable method for creatinine determination in small serum samples in the presence of proteins without chromogenes seems to be the kinetic spectrophotometric procedure. In the paediatric laboratory only small amounts of serum are available for the evaluation of the GFR, and in the case of renal failure the disturbing effect of chromogens must also be eliminated. The creatinine specific phase of the Jaffé reaction has been employed in Cook's method.

These types of creatinine assay e.g. the Galenopharm Kit, *etc.* are, however, appropriate for the quantitation of creatinine only above a serum level of 2 mg/100 ml.

Using low concentration of alkaline picrate (Bartels), the assay becomes suitable for the quantitation of creatinine below of 1.0 mg/100 ml. Using Versatol serum standards and aqueous creatinine standards (Haury, Galenopharm), comparison was made between GFR values obtained by the traditional method based on the Jaffé reaction (Popper—Mandel—Meyer) and our modification.

The new kinetic, spectrophotometric method yielded somewhat higher ($\bar{x}$ = 17 ml/min/1.73 m^2 body surface) GFR values in infants and in older children. Under the age of two years, the C_{creat} remains significantly lower ($\bar{x}$ = 48 ml/min/1.73 m^2 body surface) than C_{EDTA}, independently of the method used for creatinine determination.

ПРЯМОЙ СПЕКТРОФОТОМЕТРИЧЕСКИЙ МЕТОД ОПРЕДЕЛЕНИЯ КРЕАТИНИНА БЕЗ ПРЕДВАРИТЕЛЬНОГО ОСАЖЕНИЯ БЕЛКА ПРОБ

М. ЧЕВАРИ, И. МЕШТЯН, К. ЕБСТ

МЕЧЕКСКОЕ ГОРНОРУДНОЕ ПРЕДПРИЯТИЕ, МЕДИЦИНСКИЙ ЦЕНТР; МЕДИЦИНСКИЙ ИНСТИТУТ, ЦЕНТРАЛЬНАЯ КЛИНИКО-ХИМИЧЕСКАЯ ЛАБОРАТОРИЯ, ПЕЧ, ВЕНГРИЯ

Сообщается о непосредственном спектрофотометрическом определении креатинина в серуме и моче без белкового осаждения с применением спектрофотометра LKB—7400. Основой метода является реакция Яффе, основанная на образовании молекулярного соединения между креатинином и пикриновой кислотой в щелочной среде. Мешающее влияние других хромогенных агентов: белка, карбогидратных соединений снижается добавлением додецил-сульфата и борной кислоты, выбором времени измерения пробы, на основании изучения кинетики реакции пикриновой кислоты с белками и креатинином, а также введением «слепой пробы», т. е. измерением абсорбции реакционной смеси при кислом значении pH раствора. Содержание креатинина в пробе вычисляется из разницы измерений пробы в кислой и щелочной средах на основании калибровочной кривой или по формуле.

Методическое решение вопроса при своем простом и быстром оформлении обладает надлежащей надежностью и высокой степенью чувствительности.

VERGLEICH VON WERTEN DES GESCHÄTZTEN GLOMERULUMFILTRATS DURCH DAS SERUM-KREATININ MIT DENEN VON INULIN- UND ENDOGENEN KREATININ-CLEARANCE

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ZENTRALLABORATORIUM DES STÄDTISCHEN KRANKENHAUSES SZEGED, UNGARN

Die Werte der endogenen Kreatinin-Clearance, wurden mit verschiedenen Gleichungen nur aus dem Serum-Kreatinin gerechneten Glomerulumfiltrats mit der simultan bestimmten Inulin-Clearance verglichen. Die geschätzten Werte des Glomerulumfiltrats zeigten im allgemeinen eine gute Übereinstimmung mit der Inulin-Clearance. Die Bestimmung der endogenen Kreatinin-Clearance wird höchstens in der oberen Hälfte des Normalbereiches von Serum-Kreatinin für erforderlich gehalten; für die klinische Alltagspraxis kann man sich mit der Schätzung des Glomerulumfiltrats durch das Serum-Kreatinin begnügen.

EINE EINFACHE PHOTOMETRISCHE METHODE ZUR QUANTITATIVEN BESTIMMUNG VON PURINE IM URIN

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ZENTRALLABORATORIUM, KOMITATSKRANKENHAUS VON SZOLNOK, UNGARN

Die Untersuchung des Nukleinsäurestoffwechsels, an ihrer biologischen Bedeutung gemessen, ist — wahrscheinlich aus methodischen Gründen — ein vernachlässigtes Gebiet der klinischen Chemie. Die Frage ist — unter anderem — auch dadurch aktuell geworden, weil in der Therapie der Hyperurikämien die enzymhemmenden Stoffe in den Vordergrund getreten sind. Um den Wirkungsgrad einer solchen Therapie zu kontrollieren, ist eine Bestimmung der Menge der ausgeschiedenen Purine notwendig.

Eine Methode, die auch für die alltägliche Praxis geeignet wäre, steht noch nicht zur Verfügung.

Mit Hilfe eines koordinationschemischen Ionenaustausches haben wir eine quantitative Purinbestimmung ausgearbeitet, die auch in den klinisch-chemischen Laboratorien leicht durchzuführen ist. Das Prinzip unserer Methode ist wie folgt: Die Purine bilden mit Silber-Ionen einen wasserunlöslichen Niederschlag. Nach entsprechender Behandlung des Niederschlages geben wir zum Rest einen Kupfer-Komplex, aus dem die Silber-Ionen des Purin-Komplexes äquivalente Kupfer-Ionen freisetzen. Die freigesetzten Kupfer-Ionen sind dann photometrisch zu messen.

Die Methode ermöglicht auch die Abtrennung verschiedener Purinbasen.

KONDUKTOMETRISCHE KONTROLLE DER ELEKTROLYTKONZENTRATION DES URINS

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Vergleichende Untersuchungen der elektronischen Leitfähigkeit, der kryoskopisch bestimmten Osmolalität und der Ionenkonzentration des Urins zeigten eine gute Korrelation dieser Parameter. Leitfähigkeitsmessungen können anstelle von oder besser in Kombination mit der Osmometrie zur Ermittlung der renalen Konzentrationsleistung angewandt werden. Die elektrische Leitfähigkeit ist repräsentativ für die mit dem Urin ausgeschiedene Elektrolytmenge. Normalwerte für verschiedene Altersgruppen bei Kindern wurden ermittelt. Ein signifikanter Anstieg der Harnelektrolytkonzentration bis zum Ende des ersten Lebensjahres wurde registriert. Übereinstimmend damit fanden wir im morgendlichen Nüchternurin unter altersgerechter Ernährung einen konstanten Leitwert um $2{,}4 \cdot 10^{-2}$ Siemens (S).

THE IMPORTANCE OF ELECTRONIC, CRYOSCOPIC (THERMOELECTRIC AND EBULLOSCOPIC) OSMOLAR, AND CONDUCTOMETRIC EXAMINATION OF SERUM AND URINE IN RENAL DISEASES

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In 564 subjects the osmolar and conductometric values of fasting morning blood (0.01 ml serum) and twelve-hour urine (0.01 ml; collected from evening till morning) were measured, respectively, with a Knauer type apparatus and a home-made apparatus.

1. Measurement of the conductometric value of serum is suitable for: *a)* determining the total electrolyte content of serum and, *b)* for calculating the osmolar value, after a minimal correction (+3—5%).

2. In twelve-hour collected urine specific weight and conductivity (depending on the electrolytes, forming 40% of the specific weight) increase with the osmolar values linearly.

3. In single, daytime urines conductometry can only measure total electrolite content.

4. In acute renal failure, serum NPN and osmolality are increased. *a)* If the failure is symptomatic (hepatitis, infection, poisoning, circulatory failure)

electrolyte content and conductivity of serum and collected urine remain normal; *b)* If the cause of the renal failure is organic (pyelonephritis, gestational toxicosis) or a considerable loss of salt, then the electrolyte content, and therefore the conductivity of serum and urine decrease.

5. In chronic renal failure and in uraemia, electrolytes are excreted with the urine in excess thus the conductivity is increased.

UNTERSUCHUNGEN DER γ-GLUTAMYLTRANSPEPTIDASE-AKTIVITÄT IM URIN BEI NIERENERKRANKUNGEN

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EMIL WEIL KRANKENHAUS, BUDAPEST, UNGARN

Die diagnostische Bedeutung der Bestimmung der γ-Glutamyltranspeptidase-Aktivität (γ-GT) im Serum ist allgemein bekannt; sie wurde jedoch in der Enzymdiagnostik des Urins nicht oft angewendet. Da unter den menschlichen Organen die Niere die höchste γ-GT Aktivität aufweist, haben wir diese im Urin und Serum bei chronischen Nierenkrankheiten bestimmt, und zwar parallel mit der Kreatinin-Clearance. Für die Bestimmung wurde der γ-GT-Test der Firma Boehringer nach der von Szász empfohlenen Mikromethode benützt. Die bei unseren jungen Mitarbeitern festgestellten Normalwerte wurden mit bei Kranken gefundenen Resultate verglichen. Es konnte festgestellt werden, daß die γ-GT-Aktivität des Urins und des Serums voneinander unabhängig sind, doch steht die γ-GT Ausscheidung im Urin mit der Kreatinin-Clearance in engem Zusammenhang. Durch die erhöhte γ-GT-Aktivität im Serum wird die Ausscheidung nicht gesteigert. Bei tubulären Schädigungen und massiver Proteinurie erhöht sich die γ-GT-Aktivität im Urin.

DIE MUKOPROTEINE IM SERUM UND URIN BEI DER UROLITHIASE

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Bei 132 Ambulanzpatienten als Kontrollgruppe wurden die Mukoproteine und Proteine im Serum und Harn verfolgt. Es wurde festgestellt, daß es keine Korrelation zwischen Serummukoproteinen und Harnmukoproteinen gibt. Bei der Kontrollgruppe sind die Mukoproteine, genau gesagt das polarographisch aktive Eiweiß durch Sulphosalicylsäure unfällbar. Der Anstieg

dieses Eiweißes im Harn wird durch die Nierenerkrankung oder Harnwegenerkrankung verursacht. Bei 24 von 41 Patienten mit Nephrolithiase waren die Mukoproteine im Harn erhöht, besonders bei den klinisch schweren Fällen.

DIE BEURTEILUNG DER NIERENSTRUKTUR NACH VERSCHIEDENEN PRÄSERVATIONSVERFAHREN

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An Hundenieren wurde die Wirkung verschiedener Präservationslösungen enzymhistochemisch untersucht. Es stellte sich durch Nachweis der alkalischen Phosphatase-Aktivität heraus, daß die Zellintaktheit der proximalen Tubuli in erster Linie mit K—Mg reichen Lösungen lange gesichert werden kann. Da die spezielle Struktur der Zytomembrane und der Bürstensaum als ein hinfälliger Teil des Tubulussegments betrachtet werden kann, deutet die Bewahrung der Enzymaktivität auf die wichtige Rolle der K^+ und Mg^{++}-Ionen hin.

ANWENDUNG DER RADIALEN IMMUNODIFFUSION IN DER NEPHROLOGISCHEN DIAGNOSTIK

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Zusammenfassung nicht erhalten.

IMMUNOLOGICAL FINDINGS IN DIFFERENT TYPES OF GLOMERULONEPHRITIS

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The C^3 complement levels were investigated in relation with circulating and basal membrane connecting antibodies and those with cell-mediated immune responses in patients with histologically verified glomerulonephritis of different types.

ERFAHRUNGEN MIT DER KOMPLEMENTAKTIVITÄTSBESTIMMUNG AUS KAPILLARBLUT BEI ENTZÜNDLICHEN NIERENERKRANKUNGEN

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Es wurde eine Mikromethode zur Bestimmung des Serumkomplementes mit Hilfe von Kapillarblut entwickelt. Wir untersuchten die Gesamthämolyseaktivität des Komplements (GK) bei 12 Kindern mit akuter Pyelonephritiserkrankung, bei 12 Kindern mit akutem Pyelonephritis-Rezidiv, bei 8 Kindern mit chronischer Pyelonephritis und bei 10 Kindern mit chronischer Glomerulonephritis (GN) (bioptisch gesichert).

Wir fanden:

1. Nur die Ersterkrankungen an akuter Pyelonephritis zeigen hohe GK-Werte.

2. Akute Pyelonephritis-Rezidive boten eine ähnlich niedrige GK-Aktivität wie die chronische GN.

3. Wir vermuten eine glomerulitische Beteiligung bei der akut rezidivierenden oder chronischen Pyelonephritis.

4. Bei akuten Pyelonephritis-Ersterkrankungen sinken anfangs hohe GK-Spiegel während der (erfolgreichen) antibiotischen Therapie, zwischen den GK-Werten und dem Heilungsverlauf besteht eine negative Korrelation.

5. Die GK gesunder Probanden zeigt einen circadianen Rhythmus.

6. Wir postulieren, die GK-Bestimmung bei akuter Harnwegsinfektion im Kindesalter anzuwenden. Es gelingt hierdurch die Einschätzung, inwieweit das dem Arzt begegnende Krankheitsbild einer Ersterkrankung oder einem Rezidiv zuzuordnen ist.

DER EINFLUß DER BLOCKIERUNG DER TUBULI AUF DIE GLOMERULUSFILTRATIONSRATE

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Anh and von 1000 Untersuchungen der endogenen Kreatinin-Clearance wurde die Glomerulusfiltrationsrate mit Blockierung der Tubuli durch Paraaminohippursäure mit der ohne Blockierung verglichen. Die Differenz der Ergebnisse, ihre statistische Analyse und eventuelle Einflüsse auf die Ergebnisse werden diskutiert.

URINARY ZINC EXCRETION AND SERUM ZINC LEVELS AFTER TREATMENT WITH SALURETICS

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Urinary zinc excretion and serum zinc levels have been studied in 44 patients treated with 80 mg furosemide daily or with 100 mg hydrochlorothiazide 3 times weekly, for oedema due to congestive heart failure or other diseases of non-renal origin. Urinary zinc excretion and serum zinc levels were estimated according to Kägi and Vallee, and according to Wolff, respectively, for 3 weeks, weekly.

1. The amount of zinc excreted in urine is directly proportionate to the volume.

2. Saluretics in large dose increase the urinary zinc excretion. The benzothiadiazide compounds, in spite of their stronger saluretic effect, increase urinary zinc excretion not more than do the anthranilate compounds.

3. Parallel with the elevated zinc excretion the serum zinc level changes in dependence on the initial value.

A. If the initial level is normal, the loss of water is followed by a decrease of the serum zinc level. Till the 6—8th day the loss of zinc is slight but increases thereafter remarkably and the curve is exponential.

B. In patients with low initial serum zinc values the organism compensates the further decrease from the reserves. This compensatory mechanism comes into action without delay — the serum zinc values rise in direct proportion with time.

4. During diuretic treatment, urinary zinc is excreted in great amounts. As a result, the organism becomes poor in zinc.

FREQUENCY OF PROTEINURIA, GLUCOSURIA AND BACTERIURIA IN CHILDREN BETWEEN 4 AND 8 YEARS OF AGE

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Children were screened for proteinuria and glucosuria with the N Labstix Ames test paper and for bacteriuria with the Uricult dip test.

The frequency of proteinuria was greater than that of glycosuria (2.4% against 0.9%) at four to sixteen years. Furthermore, 4% of the girls while only 1.2% of the boys between 4 and 9 years showed bacteriuria.

DER KLINISCHE GEBRAUCH VON URICULT

Á. Jancsó, Zs. Vincze

EMIL WEIL KRANKENHAUS, BUDAPEST, UNGARN

Die Infektionen der Harnwege gehören zu den häufigsten bakteriologischen Infektionen. Nachdem sie oft ohne Symptome ablaufen, sind für deren Feststellung ausgedehnte Untersuchungen erforderlich, vor allem bei Kindern, Graviden, Alten und Diabetikern.

Uricult ist eine speziell für dieses Ziel ausgearbeitete, semiquantitative Methode, welche im Vergleich mit der klassischen Platten-Methode in der Keimzählung gleichwertige Ergebnisse gibt.

Wir haben mit der Uricult-Methode Keimzahl-Definitionen bei verschiedenen Harnweg-Erkrankungen in 150 Fällen gemacht. Die Resultate wurden von verschiedenen Gesichtspunkten verarbeitet und erörtert.

ERFAHRUNGEN MIT DER URICULT METHODE

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An 200 Urinproben wurde die Zuverlässigkeit und Reproduzierbarkeit eines Schnellverfahrens zur quantitativen Keimzahlbestimmung im Urin geprüft. Der Vergleich mit dem Gußplattenverfahren ergab gute Übereinstimmung. Die Anzahl der falschpositiven Ergebnisse war 6 in den geprüften 200 Fällen.

ANWENDUNG EINES URIN-BAKTERIOLOGISCHEN SCHNELLVERFAHRENS

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PÄDIATRISCHES AMBULATORIUM UND KINDERSPITAL BUDA, BUDAPEST, UNGARN

Mit dem Uricult-Schnelltest (Orion) wurden Urinbakteriologische Untersuchungen bei 0—14 jährigen Kindern mit Harnwegsinfektionen im Krankenhaus und ambulant durchgeführt. Parallel mit dem Schnelltest wurde in jedem Fall auch eine Keimzählung vorgenommen. Die Urinproben waren: Mittelstrahlurin; steril gesammelter Urin; mit Blasenpunktion gewonnener Urin; Katheterurin.

Die Inkubationszeit war 24 Studen bei Zimmertemperatur oder bei 37° im Thermostat.

Die Resultate des Schnelltests stimmten mit den Keimzählungen überein, und erlaubten eine erfolgreiche Kontrolle der Therapie und der Rezidiven.

EIN NEUES VERFAHREN ZUR BAKTERIOLOGISCHEN KEIMZAHLBESTIMMUNG IM HARN

I. Miczbán, L. Zsoldos

JÁNOS KRANKENHAUS, BUDAPEST, UNGARN

In 100 frisch gewonnenen Harnproben wurden Keimzahlbestimmungen mit dem von Hammer modifizierten klassischen Standardverfahren und mit Uricult parallel durchgeführt. Die vergleichenden bakteriologischen Untersuchungen bewiesen, daß Uricult als eine semiquantitative Kulturmethode mit dem Standardverfahren gleichwertig und für qualitative Schätzung entsprechend ist. Die auf dem Uricult-Medium gewachsenen Kolonien sind für weitere bakteriologische Untersuchungen zwecks eingehender Differenzierung, Identifizierung und Resistenzbestimmung geeignet. Bei entsprechender Kollaboration mit dem Kliniker wird durch den Uricult-Test die Überbelastung des Laboratoriums vermindert und die Keimzahlbestimmung im Urin exakter gestaltet.

KEIMZAHLBESTIMMUNG IN URIN UND GALLE MIT HILFE VON URICULT®

E. Krompecher

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Die Bakterienzahl in Urin und Galle wurde mit direkter Keimzählung, mit biochemischen Reaktionen und mit dem Uricult-Test bestimmt. Die Ergebnisse der direkten Keimzählung und des Uricult-Tests zeigten eine gute Korrelation.

Die Vorteile des Uricults sind wie folgt:

1. Die Keimzahl kann semiquantitativ festgestellt werden.

2. Wenn die Verarbeitung des entnommenen Materials nach längerer Zeit erfolgt, kann es vorkommen, daß sich sichtbare Kolonien auf den Uricult-Platten bilden.

3. Auf den URICULT-Platten kann die Keimzahl auch unter einem Wert vom 10^5/ml semiquantitativ leicht beurteilt werden.

Die Nachteile der URICULT-Methode ist, daß bei einer Keimzahl über 10^5/ml isolierte Kolonien nur selten gewonnen werden können.

DIE KEIMZAHL DER B-GALLE BEI DEN ENTZÜNDLICHEN ERKRANKUNGEN DER GALLENWEGE

GY. DÓBIÁS, É. KROMPECHER

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Die Keimzahl der B-Galle wurde bei entzündlichen Gallenwegserkrankungen untersucht. Die Ergebnisse wurden mit der Keimzahl der B-Galle solcher Kranken verglichen, bei denen entzündliche Erkrankungen der Gallenwege auszuschließen waren. Die Bedeutung der Keimzahl der Galle wird hervorgehoben.

КЛИНИЧЕСКАЯ ОЦЕНКА БАКТЕРИОЛОГИЧЕСКИХ АНАЛИЗОВ НЕКОТОРЫХ ИНФЕКЦИЙ МОЧЕВЫВОДЯЩИХ ПУТЕЙ

К. САБО, В. РАНКО, Ю. РОШТАШ

МЕДИЦИНСКАЯ СЛУЖБА ВЕНГЕРСКОЙ НАРОДНОЙ АРМИИ, БУДАПЕШТ, ВЕНГРИЯ

Часто хронический пиелонефрит определяют у больного уже в стадии почечной недостаточности, а нередко — только на вскрытии. Наряду с разнообразием клинических проявлений заболевания причиной этого является то, что многие методы лабораторных исследований не всегда пригодны для достоверного обнаружения пиелонефрита в начальной стадии или же для обнаружения болезнетворного агента.

Для установления бактериологического диагноза авторы наряду с традиционными методами культивирования и подсчета бактерий применяли способ обогащения.

В докладе описаны эти бактериологические методы и дано сравнение полученных результатов с результатами других исследований, а также — с клинической картиной заболевания.

A NOVEL, CONVENIENT, COMBINED RAPID BIOCHEMICAL AND CONFIRMATORY QUANTITATIVE TEST SYSTEM FOR THE DETECTION OF URINARY TRACT INFECTIONS

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AMES-MILES CO., MÜNCHEN, GERMAN FEDERAL REPUBLIC

The paper describes a newly devised, miniaturized, stable test system designed to detect viable bacterial populations in the range of 10^1 through 10^5 and higher. The results in a clinical study of approximately 700 urines will be reported.

The test system consists of three stable reagent zones attached to plastic strip. Each zone contains bacterioreactive ingredients within a paper matrix. One reactive zone provides a rapid (30 sec) qualitative biochemical test for bacteriuria. The remaining two zones are designed to support bacterial growth and simultaneously provide the bacterial population by means of bacterial "location" density. One growth zone is selective for Gram negative bacteria, while the other provides an index of the total (Gram negative and Gram positive) bacterial populations in the urine. The test is performed by dipping the strip into the well-mixed clean catch, mid-stream urine specimen. The biochemical reactive zone is read within 30 seconds. The "inoculated" test strip is then placed in a plastic pouch and incubated at 37 °C. The results provide confirmatory quantitative results in bacteriuric specimens.

DIAGNOSIS OF ASYMPTOMATIC PYELONEPHRITIS

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Abstract not submitted.

ANTIBIOTIKUMEMPFINDLICHKEIT UND DIAGNOSTIK DER AUS URINISOLIERTEN ENTEROBACTERIACEAEN

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Die grobquantitative Keimzählung aus 10 μl Urin nach Hussels ergab vorwiegend Enterobacteriaceaen. Zur genauen Keimdifferenzierung wurden

die Plättchendiffusionsmethode nach Lányi und Molnár und Schnellteste angewandt.

Die Empfindlichkeit gegen Nalidixsäure wurde mit der BIOTEST® Papierscheibenmethode untersucht.

Die in den Jahren 1963—1973 gewonnenen Ergebnisse werden besprochen.

DETERMINATION OF THE ANTIBIOTIC LEVEL IN THE HUMAN KIDNEY

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Determination of the blood and urinary antibiotic level is a generally accepted method. It is known that some antibiotics reach a higher and others a lower level in urine than in blood. In pyelonephritic processes it would seem important to know whether the antibiotics have reached the therapeutic concentration in renal tissue.

We have studied the level of gentamicin in kidneys obtained from five patients in the final stage of renal disease. The patients had received 240 mg gentamycin intramuscularly 2 hours before nephrectomy. The excised kidneys were homogenized and the gentamycin level was determined by a microbiological method using agar diffusion. Staphylococcus aureus ATCC 6538P served as the test bacterium. The antibiotic level in blood and urine were determined also. The results showed a bactericidal concentration of gentamycin not only in blood and urine but also in the kidneys, but the kidney level was lower than the urinary one.

ERFAHRUNGEN UND ERGEBNISSE MIT DEM EINFACHEN UND KOMBINIERTEM TTC-TEST IN DER DIAGNOSTIK DER SIGNIFIKANTEN BAKTERIURIE

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Prinzip und Methodik des einfachen und des modifizierten, für antibiotische Resistenzbestimmungen geeigneten TTC-Tests werden besprochen.

Die Ergebnisse einer Serien- und Reihenuntersuchung mit Hilfe dieser Methoden an den am meisten gefährdeten Gruppen der Population werden geschildert.

RELATIONSHIP BETWEEN THE KIDNEYS AND LIVER IN DRUG METABOLIZING ENZYME INDUCTION

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The activity of drug metabolizing enzymes is higher in the liver than in other tissues. The relationship of the activities in various tissues may be of physiological importance. To test this possibility, two experimental models have been evaluated.

1. Activity and inductibility of O-demethylase (O—DM) in experimental kidney injury: was estimated in 9000 g supernatant of rabbit liver and renal cortex, after the administration of mersalyl in a dose of 4 mg Hg/kg subcutaneously. Phenobarbital in a dose of 25 mg/kg was used as an enzyme inductor. The serum urea and creatinine levels increased after mersalyl administration while O—DM activity decreased considerably in the renal cortex. Phenobarbital induced the activity of O—DM, but mersalyl depressed the inductibility of O—DM. In the liver, O—DM activity did not change significantly, due probably to the increased concentrations of urea and creatinine. The inductibility of O—M increased after phenobarbital administration.

2. Activity and inductibility of O—DM in experimental liver injury was studied after production of fatty liver by hydrazine given in doses 0.5 mmoles/kg. Hydrazine did not cause liver necrosis or kidney damage. Phenobarbital was used for the induction of O—DM. The analytical procedures were similar to those in the renal model. Under these conditions, marked fatty liver developed. O—DM activity decreased in the liver and the inductibility was lower than in the control animals. In the kidney, hydrazine increased both the basal activity and the inductibility of O—DM.

It is concluded that in liver injury the kidney increases the activity and inductibility of its drug metabolizing system. On the other hand, in kidney injury, the liver substitutes its activity and inductibility. Thus, there is a relationship, in the detoxication mechanisms of the kidney and the liver. They supplement each other and this supplementation may be of importance under physiological and particularly under pathological conditions.

LABORATORY EXAMINATIONS IN EPIDEMIC HEPATITIS

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For the diagnosis of epidemic hepatitis the following clinico-chemical and immunoserological tests are used: serum bilirubin, thymol turbidity, serum proteins, gamma-globulin, GOT, GPT, alkaline phosphatase, serum cholesterol, bile acids, latex test, and Australia antigen. For differential-diagnostic purposes the following tests are applied: alpha-HBDH, total-lactate-dehydrogenase, profile-LDH, gamma-GT, blood ammonia, serum iron, total iron binding capacity, transferrin saturation, serum copper, serum magnesium, haemoglobin and haematocrit. After admission of the patient, the above twenty-two tests were done once weekly. It was found that the elevated magnesium level, in relation to cholesterol, was valuable for the judgement of cholestasis associated with viral hepatitis. The blood ammonia and serum magnesium levels, too, are of value in differential diagnosis. The values for serum iron, copper, serum gamma-globulin, and the latex test are moderately useful for differential-diagnostic purposes.

COMPARATIVE STUDIES OF ENZYMATIC ACTIVITY IN HEPATOBILIARY DISORDERS

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JÁNOS HOSPITAL, BUDAPEST, HUNGARY

In patients with hepatitis, liver cirrhosis, or obstructive jaundice the activities in the serum of the following enzymes were determined simultaneously: glutamate-pyruvate transaminase, glutamate-oxalate transaminase, guanase, alkaline phosphatase, alanine-arylamidase, gamma-glutamyl transpeptidase and alpha-amylase. Of these, the activities of glutamate-pyruvate transaminase and guanase, proved the most reliable for the differentiation of hepatocellular jaundice and obstructive jaundice. The activity of gamma-glutamyl transpeptidase of endothelial localization is a very sensitive indicator of pathological hepato-biliary processes; its activity is considerably increased in hepatic as well as in biliary diseases.

FRACTIONATION OF HUMAN SERUM AND URINARY CHOLINESTERASE (ACYLCHOLINE-ACYLHYDROLASE E.C.3.1.1.8.) ISOENZYMES WITH POLYACRILAMIDE-GEL-GRADIENT ELECTROPHORESIS

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Cholinesterase activity is linked with proteins of different electrophoretic mobility. Studies published so far carried out electrophoresis on homogeneous pore size media. For the separation of heterogeneous human cholinesterase of extreme molecular size, polyacrylamide pore-gradient electrophoresis provides more advantageous conditions than other procedures. By means of gradient gel columns, in spite of the shorter separation time the slowly migrating fractions may be separated adequately and the rapid fractions do not fall away from the gel columns.

Polyacrylamide electrophoresis on monotone and gradient gels has been applied for studying human serum and urinary proteins and cholinesterase isoenzymes in various conditions especially renal diseases. The severity of renal malfunction may be assessed by the electrophoretic technique.

γ-GLUTAMYLTRANSPEPTIDASE ACTIVITY IN LIVER DISEASE

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γ-GT activity was investigated in patients with liver disease and in control subjects. In every case the activities of SGOT, SGPT, LDH, AP enzymes and the bilirubin level were also estimated. The clinical significance of γ-GT enzyme activity is discussed.

EFFECT OF IMMUNOSUPPRESSIVE THERAPY ON THE HUMORAL IMMUNOLOGICAL ALTERATIONS IN CHRONIC AGGRESSIVE HEPATITIS

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The concentrations of IgG, IgA, IgM serum coeruloplasmin, alpha-2-macroglobulin, beta-1-C-globulin and transferrin were determined by radial immunodiffusion in patients with chronic aggressive hepatitis before and after immunosuppressive therapy. In addition, the serum alpha-fetoprotein level was estimated by counterelectrophoresis and the antinucleoprotein factors by the rapid LE test (Hyland). After treatment, the concentration of immunoglobulins, especially of IgG, was decreased. In some cases the other serum glycoproteids, too, displayed a decreasing tendency.

BLOOD COAGULATION DISTURBANCES AND LIVER INJURY INDUCED BY LANTHANUM TRICHLORIDE (La)

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The rare-earth metals are well-known to exert an anticoagulant effect, due to complex-formation with some blood clotting factor. Their specific activity on the liver is also well-known. In view of the close connection between liver and haemostasis, the effect of La on blood coagulation has been studied with especial consideration to the liver injury.

The examinations were carried out on rabbits. As the action of lanthanum depends on its dose, we examined the effect of a single large dose and of repeated smaller doses. The effect of a single dose caused in most animals an acute severe haemorrhagic diathesis, with a significant decrease of the prothrombin complex. In the animals treated chronically the findings were quite different. Beside the increase of fibrinogen level a decrease of prothrombin consumption was characteristic. This pointed to a disturbance of factor VIII production or to an impaired platelet function.

BETRACHTUNGEN ZUR BESTIMMUNG DES SERUMCHOLESTERINS

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Im klinischen Routinelaboratorium haben sich spektrophotometrische Bestimmungsmethoden durchgesetzt, die zur Erhöhung der Präzision und zur Beschleunigung des Arbeitsablaufes die Zahl der Arbeitsschritte niedrig halten. Technisch besonders einfach ist die Arbeitsweise nach Huang et al. [1], die lediglich zwei Pipettierungen erforderlich macht. Die bei diesem Verfahren auftretende große Reaktionswärme bewirkt jedoch im Serumansatz eine Beeinträchtigung der Spezifität der Aussage. Ferner wird die Analysenrichtigkeit dadurch verringert, daß die üblicherweise aus Cholesterin in Eisessig bestehende Standardlösung mit dem Farbreagens anders reagiert als das Serumcholesterin.

Unsere Untersuchungen [2, 3] zeigten, daß eine exzessive Erwärmung des Reaktionsansatzes besonders bei der Verwendung großer Ansatzvolumina zu erwarten ist. Es gilt zu verhindern, daß die Reaktionstemperatur während der Umsetzung eine bestimmte kritische Schwelle, die bei ca. 25 °C liegt, überschreitet. Bei mittleren Ansatzmengen (2.0 ml Farbreagens + 0.05 ml Serum) geschieht dies in der Weise, daß die Serumproben mit einem auf +4 °C unterkühltem Farbreagens versetzt wird. Um eine Vorstellung über den durch eine allfällige Überschreitung der kritischen Temperatur verursachten Fehler zu gewinnen, haben wir die Bestimmungsreaktion bei verschiedenen Reaktionstemperaturen studiert. Dabei stellten wir fest, daß der Einsatz eines z.B. 25 °C aufweisenden Farbreagens zu einem durch »Nichtcholesterinchromogene« verursachten positiven Fehler von 40 mg Cholesterin/100 ml führen kann. Die Richtigkeit der Analysenresultate konnte durch die Einführung einer »serumähnlichen« wässerigen Cholesterinstandardlösung wesentlich verbessert werden. Der von uns empfohlene Cholesterinstandard ist eine durch Einwaage bereitete klare und stabile Lösung von Cholesterin in Wasser.

Als wichtigster Nachteil aller auf der Liebermann—Burchard Farbreaktion beruhenden Cholesterinbestimmungsmethoden gilt ihre mäßige Empfindlichkeit.

Es bleibt zu hoffen, daß die in letzter Zeit vorgestellten kinetischen Meßmethoden und die enzymatischen Verfahren diesbezüglich einen Fortschritt in die Analytik der Cholesterinbestimmung bringen werden.

[1] HUANG, T. C. et al., Anal. Chem. **33,** 1405 (1961).
[2] VASS, L.: Schweiz. med. Wschr., **102,** 914 (1972).
[3] VASS, L.: Clin. chim. Acta, **45,** 313 (1973).

SCREENING FOR SERUM LIPOPROTEID FRACTIONS

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The serum lipoproteid fractions have been studied in 250 patients by means of agarose-gel electrophoresis. In 200 cases the serum lipoproteid (LP) fractions showed a normal distribution (no chylomicron fraction; alpha-LP, 23%; beta-LP, 53%; and prebeta-LP, 24%), in accordance with data in the literature. In 50 cases pathologically increased prebeta fractions were detected. These did not seem closely connected with aging, and generally occurred in hyperlipaemic patients. The hyperlipaemia mostly belonged to type No IV of Fredrickson et al. The patients had been admitted with undetermined complaints and the metabolic disorder which had not yet manifested in any organic disease was revealed by their screening for lipoproteid fractions.

MEASUREMENT OF SERUM TOTAL LIPOID LEVELS

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Recently the serum total lipoid level is widely measured applying test kits, the principle of which is based on the sulpho-phospho-vanillin (SPV) method of Zöllner and Kirsch (1962) without lipid extraction. Normal and pathological sera were investigated by the aid of different test kits (Boehringer, Reanal, Galenopharm) and by using the SPV method after extraction of lipids. The total lipoid level showed significant differences in dependence on the test applied. Specificity of the SPV reaction was greater after lipid extraction especially in the pathological cases, so that lipid extraction is recommended.

THIN-LAYER AND GAS-LIQUID CHROMATOGRAPHIC LIPID ANALYSIS OF SERUM AND BIOPSY SAMPLES

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Disturbances of lipid metabolism may occur in the form of an independent clinical picture or as a concomitant of some illness. For assessing the lipid disturbance it is usual to analyse serum samples. The main lipid fractions

(triglyceride, free fatty acid, cholesterol, phospholipid) are usually measured spectrophotometrically, and the lipoproteins are separated electrophoretically.

In certain metabolic disturbances (e.g. diabetes mellitus) the serum lipid level is high and the accumulation of fat in the liver is also increased. In others, the lipoid content of the liver may be high without being indicated by the serum lipid level.

Accordingly, it is of importance to examine the lipid fractions of the serum as well as of the tissues with more sensitive methods. Our results obtained by lipid extraction, thin-layer chromatographic and gas-liquid chromatographic separation of lipid classes and their fatty acids has confirmed the importance of the tissue lipid composition for the clarification of metabolic disorders.

A case of a variety of Niemann-Pick's disease in a child is reported.

COMPARATIVE EXAMINATION OF IRON DETERMINATION

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The common methods of iron determination were compared as to their labouriousness, reproducibility and reliability, taking into consideration the sensitivity difference arising from the different molar extinction coefficients of colour reagents.

A detailed report is given of the results obtained with the method applying Ferrozin (3-)2-pyridyl(-5, 6-bis)4-phenylsulphonic acid(-1, 2, 4,-triazine) as the colour reagent.

IRON DEFICIENCY AND ITS THERAPEUTIC POSSIBILITIES IN PREGNANCY

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Iron deficiency has been studied in late pregnancy by determination of serum iron content, iron binding capacity, serum copper, serum magnesium, serum proteins, haemoglobin and haematocrit values. It was found that haemoglobin and haematocrit values are insufficient to predict iron deficiency.

A latent iron deficiency may exist when the haemoglobin and haematocrit values are still normal.

Different iron therapies were compared and it was found that reduced iron + ascorbic acid + calcium lactate + copper sulfate taken after meals was a suitable therapy of pregnancy hyposiderosis.

GRAPHISCHE DARSTELLUNG DER VERÄNDERUNGEN WICHTIGER PARAMETER WÄHREND DER NORMALSCHWANGERSCHAFT

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Die Normalwerte einiger Laboratoriumsuntersuchungen bei Normalschwangerschaft und nicht schwangeren Frauen wird graphisch gegenübergestellt. Die graphische Darstellung wird als Hilfsmittel bei Bewertungsproblemen der Laboratoriumsuntersuchungen bei Schwangeren empfohlen.

BESTIMMUNG DER KOMPLEMENTAKTIVITÄT IM SERUM — EINE EINFACHE MIKROMETHODE FÜR SERIENMÄSSIGE ANALYSEN AUS KAPILLARBLUT

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Die Methode gestattet Komplementaktivitätsbestimmungen aus 10 μl Kapillarblut-Serum mit ca 3 Minuten Arbeitsaufwand je Analyse in der Serie. Sie ist nicht aufwendiger, als eine einfache klinische-chemische Analyse. Sie ist geeignet zur Automatisierung. Der Variationskoeffizient liegt unter 3%. Prinzip der Methode ist die Einwirkung des Serums auf ein haemolytisches System (Hammelerythrozytensuspension im Gemisch mit einem Antihammelerythrozytenserum) unter standardisierten Bedingungen und anschliessender photometrischer Bestimmung des durch Erythrozytolyse freigesetzten Haemoglobins. Dessen Konzentration ist anhand einer Eichkurve das Mass für die Komplementaktivität des Serums. Ein im Analysengang mitgeführter Bezugsstandard eliminiert methodische Schwankungen.

Man benötigt (selbst herstellbare) Hammelblutkonserven, Barbitalpuffer, Kaliumsulfat-Borsäure-Lösung, ein Haemolysin gegen Hammelerythrozyten, Transformationslösung, einen (selbst herstellbaren) *Standard*normalserumpool, sowie ein Eppendorf-Mikrolitersystem mit Photometer.

Die Komplementaktivität frischen Serums läßt sich durch Mischung mit Kaliumsulfat-Borsäure-Lösung bis zu 3 Monaten stabilisieren und bei 4 °C bis zur Analyse aufbewahren. Da Hammelblutkonserven auch 3 Monate haltbar sind, ist die Eichkurve 1/4-jährlich zu ermitteln. Eine Analyse erfordert 4 Pipettierungen. Das Ergebnis erhält man unmittelbar in % der Norm (2 S — Bereich: 76—124%).

LITERATUR

DROGIES, I. und G. HAUSCHILD: Dtsch. Ges. wesen **29**, 1126 (1964).

CLINICAL IMPORTANCE OF THE SERUM LITHIUM LEVEL

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Serum lithium levels were studied by flame photometry during lithium therapy of psychiatric patients. In 1524 consecutive serum specimens of 248 patients, in 44% lithium was in the therapeutic range, in 47% below this optimum concentration, and in 2% at toxic levels. 10% of the samples contained no lithium.

These results suggest that a prerequisite of a successful and harmless lithium therapy are regular lithium monitoring and appropriate clinical evaluation of the results. The use of lithium carbonate requires a close cooperation between psychiatrist and the clinical laboratory.

ESTIMATION OF SERUM LITHIUM BY FLAME PHOTOMETRY

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A method of serum lithium assay has been worked out, applying a Carl Zeiss Jena Model III flame photometer. The reagents are as follows. Stock lithium solutions (SLS—0, SLS—10, SLS—20); each solution contains 1400 mEq/l sodium, 45 mEq/l potassium, 50 mEq/l calcium as well as 10 and 20 mEq/l lithium (from $Li_2SO_4 . H_2O$) in SLS—10 and SLS—20, respectively. (Storage in plastic bottles and in a cold place).

Working lithium standard solutions (WLS—0, WLS—1, WLS—2); the stock solutions are diluted 1 : 10 by demineralized water. (To be prepared every two weeks; storage as above).

Preparation and analysis. One ml of each of the solutions WLS—0, WLS—1, WLS—2 is diluted with 20.0 ml of demineralized water. The apparatus is adjusted to scale division 180 with the diluted WLS—1 (air pressure 0.34 at., acetylene pressure 64 water mm, interference filter for 671 nm). One ml serum is diluted with 20.0 ml demineralized water. The diluted samples are measured directly without deproteinization in the adjusted instrument. Calculation as usual. Accuracy of the method is adequate for clinical use.

ANALYSIS OF FACTORS IN LITHIUM DETERMINATION BY FLAME PHOTOMETRY

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Chemical and physical factors influencing serum lithium determinations were studied by a Carl Zeiss Jena Model III flame photometer. A linear relation was found between emission at 671 nm and the lithium concentration in both lithium-containing sera and aqueous lithium solutions. Lithium emission decreased in aqueous lithium solutions of pH 2 and less. Sodium, potassium and calcium significantly increased the galvanometer readings. A so-called "serum effect" increasing the meter readings was observed.

CHEMIKALIEN-TESTSETS — EINE WIRKSAME RATIONALISIERUNG DER ARBEIT IM KLINISCHEN BIOCHEMISCHEN LABORATORIUM

J. Gregorek

LACHEMA, BRNO, TSCHECHOSLOVAKEI

In allen Staaten mit entwickelten Gesundheitswesen steigt die Anzahl der biochemischen Untersuchungen sowohl wie die Forderungen betreffs Effektivität der Laboratorien. Die biochemischen Testsets können eine bedeutende Zeitersparung bringen, besonders bei der Herstellung von Arbeitslösungen. Sie sind auch für die Präzisierung der Arbeit und für die Standardisierung der Methoden vom Nutzen. Die Erfahrungen tschechoslowakischer Laboratorien werden anhand von konkreten Ziffern erörtert.

QUALITY CONTROL

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Abstract not submitted.

SOME USES OF THE PROGRAMMATED CALCULATOR IN THE CLINICAL LABORATORY

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The modern clinical laboratory meets the requirements, to perform reliable tests of a great number daily. These assays are usually carried out in the chemical laboratory where specimens of blood, urine, cerebrospinal fluid, and tissues are examined.

Although many sophisticated analytical instruments are available the complexity of the tested material on the one hand, and the minute concentration of some constituents on the other, require ingenuity and skill from the clinical chemist to cope with the task.

The known chemical constituents of the blood and urine are large, the tests routinely performed in the clinical-chemical laboratory are relatively small.

The development and introduction of calculators has made possible the significant increase of efficiency in data handling by the laboratory. The purpose of the lecture is to discuss some of the applications of the calculator in the clinical-chemical laboratory, *e.g.*

Calculation of percent of protein fractions;

Calculation of concentration of fractions based on the spectrophotometric data gained by an Autoanalyzer.

AMYLASE-BESTIMMUNG

J. Fischer

LACHEMA, BRNO, TSCHECHOSLOVAKEI

Die Probleme der spektrophotometrischen Bestimmung der Amylase und die optimalen Bedingungen der Standardisierung der Methode werden besprochen.

PROFIL LDH-UNTERSUCHUNG BEI HERZINFARKT

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KOMITATSKRANKENHAUS, SALGÓTARJÁN, UNGARN

Die LDH-Aktivität im Serum setzt sich aus den Aktivitäten von mindestens 5 verschiedenen Isoenzymen zusammen, die sich durch Elektrophorese trennen lassen. Fraktion LDH—1 stammt zum größten Teil aus dem Herzmuskel und den Erythrozyten, während Fraktion LDH—5 hauptsächlich aus Leber und Skelettmuskulatur.

Die photometrische Bestimmung dieser Isoenzyme ergibt zwei Extinktionen; die Extinktion des LDH—1 Isoenzym dividiert durch die Extinktion des LDH—5 Isoenzyms ergibt den Profil-LDH-Quotienten. Das normale Verhältnis: 0.8—1.2. Wenn der Quotient kleiner als 0.8 ist, ist der Wert von LDH—1 höher, und wenn der Quotient größer als 1.2 ist, weist er auf die Erhöhung von LDH—5 hin.

Mit Hilfe der Profil-LDH (Gödecke) wurde der Zusammenhang zwischen Herzinfarkt und Profil-LDH Quotient nach Babson und Emerson erforscht.

KINETISCHE MESSUNG DER ENZYMAKTIVITÄTEN BEI OPTIMIERTEN BEDINGUNGEN

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KLINISCH-CHEMISCHES ZENTRALLABORATORIUM DER MEDIZINISCHEN UNIVERSITÄT PÉCS, UNGARN

Die Aktivität von GOT, GPT, LDH, alfa-HBDH, AP und gamma-GT wurde mit dem Reaktionsgeschwindigkeitsanalysator LKB 8600 bei optimierten Bedingungen in Seren von Patienten und gesunden Personen bestimmt. Bei den einzelnen Enzymbestimmungen wurden die optimierten Werte mit den Resultaten der konventionellen Verfahren verglichen. Weiterhin wird über eine optimierte standardisierte kinetische Routinemethode berichtet, womit die obigen Enzymaktivitäten auch bei nicht automatisierter Ausrüstung gemessen werden können. Die Messergebnisse wurden mit den optimierten Testergebnissen verglichen.

CHANGES OF THE HBDH/LDH RATIO AFTER UROLOGICAL OPERATIONS

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The postoperative ECG-s often show reversible changes in repolarization. After different urological operations, the LDH and HBDH levels were determined over a period of three to four days under ECG control. In a few patients with postoperative ECG changes, the HBDH/LDH ratio increased, but in most of them it remained unchanged. If the ECG showed no hypoxic damage to the myocardium, the HBDH/LDH ratio decreased.

BLASTTRANSFORMATION DER LYMPHOCYTEN BEI STRAHLENBEHANDELTEN PATIENTEN

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ZENTRALLABORATORIUM DES TÉTÉNYI KRANKENHAUSES, BUDAPEST, UNGARN

Bei 50 Patienten, die wegen tumoröser Erkrankungen einer Strahlenbehandlung unterzogen wurden, führten wir neben den hämatologischen Routineuntersuchungen (Blutsenkung, qualitatives und quantitatives Blutbild und Sternalpunktat) auch den Lymphocyten-Transformationstest nach Rabinowitz durch. Noch bevor die Routinen Untersuchungen Veränderungen zeigten, war schon eine signifikante Herabsetzung der Zahl der Blasten zu verzeichnen. Im Gegensatz zu dem normalen Prozentsatz von 70—80, war die Zahl der transformierten Lymphocyten bei den bestrahlten Patienten zwischen 50—60 %. Der Test scheint zur Kontrolle der ambulanten Kranken in Hinblick auf die Vorbeugung von Komplikationen (Verhinderung einer kritischen Insuffizienz des Immunsystems) geeignet zu sein.

TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORETIC ANALYSIS OF NUCLEAR PROTEINS OF NORMAL AND LEUKAEMIC LYMPHOCYTES

M. Kellermayer, K. Jobst, H. Busch

INSTITUTE OF CLINICAL CHEMISTRY, UNIVERSITY MEDICAL SCHOOL, PÉCS, HUNGARY, AND DEPARTMENT OF PHARMACOLOGY, BAYLOR COLLEGE OF MEDICINE, HOUSTON, TEXAS

A two-dimensional polyacrylamide gel electrophoretic method has been worked out. In the first dimension the proteins run according to their charges and in the second sodium-dodecyl-sulphate gel according to their molecular

size. By this method three or four times as many proteins could be separated from saline soluble extracts of human leukaemic and normal lymphocyte nuclei as by one-dimensional disc gel electrophoresis. Although the similarities of separated proteins were striking, several differences were observed.

With its high resolution and reproducibility two-dimensional gel electrophoresis provides much information on the protein composition of organelles of cells, human serum and urine in different pathological conditions.

DIE BEDEUTUNG DER HARNSÄUREBESTIMMUNG UND IHRER SCHWIERIGKEITEN

V. A. Velősy

ZENTRALLABORATORIUM, KOMITATSKRANKENHAUS. SZOLNOK, UNGARN

Außer der Gicht gibt es zahlreiche Zustände, bei denen die Harnsäure eine wichtige Rolle spielt. Unter anderen sind der Diabetes, die arteriellen Verschlußkrankheiten, Übergewicht, Schwangerschaftstoxikose, Hypertonie und verschiedene Nierenkrankheiten zu erwähnen. Chemisch ist die Harnsäure in drei Tautomer-Formen bekannt, in Trilaktim-, Dilaktim- und Laktam-Form; im menschlichen Organismus kommt allein die Laktam-Form vor.

Dementsprechend ist das Uricase auf diese Form spezifisch. Die synthetischen Präparate enthalten dagegen die drei Formen in unbekannter Verteilung. Da die verschiedenen Tautomere unterschiedliche Reduktionswirkungen haben, muß auch bei den Reduktionsmethoden und bei den enzymatischen Verfahren mit einer nicht zu vernachlässigenden Fehlerquelle gerechnet werden. Die Verwendung von verschiedenen Präparaten als Standard, kann zu mehr oder weniger divergenten Resultaten führen.

THE NORMAL RANGE OF SERUM URIC ACID

I. Horvátth

BAJCSY-ZSILINSZKY HOSPITAL, BUDAPEST, HUNGARY

Abstract not submitted.

A NEW METHOD (EYETONE-DEXTROSTIX) FOR RAPID BLOOD GLUCOSE DETERMINATION

A. WAGNER

AMES-MILES CO., MÜNCHEN, GERMAN FEDERAL REPUBLIC

A new efficient method is reported that offers the convenience of performing blood glucose determinations in the physician's office, at the patient's bedside, and in the hospital out-patient department. The procedure presents a new analytical approach to blood glucose measurement.

The method utilizes an electro-optical system for measuring the degree of colour development on reagent strips in reaction to a drop of whole blood. The amount of light reflected from the reagent area of the strip is measured and a direct readout of blood glucose concentration is provided on the meter scale of the instrument.

The ease of operation and convenience of the system allows the clinician to take advantage of prompt definition of blood glucose concentration. Use of the system minimizes the time required for specimen transport and data transmission.

METEOROLOGICAL FRONT ACTIVITY AND CLINICO-CHEMICAL PARAMETERS

K. FENDLER, M. ROMHÁNYI

CENTRAL LABORATORY OF THE COUNTY HOSPITAL, PÉCS, HUNGARY

In earlier examinations it was found that the blood haemoglobin levels of hospitalized "normal" patients changed during front activity. In the present work, blood urea nitrogen, serum potassium and sodium levels have been studied during meteorological front activity. In the lack of front activity, the values were normal. During strong activity, they displayed remarkable changes, the direction of which depended upon whether the front was warm or cold.

AUFGABE, ROLLE UND MÖGLICHKEITEN DES MEDIZINISCH-CHEMISCHEN LABORATORIUMS IN DER NEPHROLOGISCHEN PROPHYLAXE

J. Faller

STÄDTISCHES LABORATORIUM, SZÉKESFEHÉRVÁR, UNGARN

Ein Bestreben für aktive und erfolgreiche Prophylaxe auf dem Gebiet der Nephrologie ist überall bemerkbar seitdem die Wichtigkeit der signifikanten und asymptomatischen Bakteriurie und damit die der Fürsorge der potentiellen Nephrotikern erkannt worden ist. Es ist ein Prinzip geworden, daß alle Personen mit signifikanter Bakteriurie als potentielle Nephrotiker anzusehen sind.

Der Nachweis einer signifikanten Bakteriurie ist eine bakteriologische Aufgabe und es gibt bereits Methoden, die für Massen- und Serienuntersuchungen brauchbar sind.

Die Möglichkeiten ihrer Anwendung werden eingehend diskutiert.

A NEW MEETING IS ANNOUNCED BY HOLLAND ORGANIZING CENTRE

Name: 5th Congress of the European Society of Pathology
Type: Scientific medical congress
Date: 5—10 October, 1975
Place: Vienna, Austria
Building: Hofburg Congress Centre
Organizing body and/or sponsor: the European Society of Pathology
Secretariat: c/o Holland Organizing Centre, 16 Lange Voorhout, The Hague, The Netherlands
Subject: pathology
Languages used: preferably English
Simultaneous interpretation: no
Attendance (est.): 500
Free (Limited) On invitation only
Number of papers: ca. 180
Free (Invited)
Language of papers: English
Deadline for abstracts: 1 March, 1975
Deadline for complete texts: not applicable
Abstracts will be published in: book form
Date of publication: 15 September, 1975
Full papers will be published in: not applicable
Date: —
Price: —
Obtainable from: —
Exhibition: Yes
Number of exhibitors (est.): 30
Subject: technical and scientific; medical equipment, pharmaceuticals
Information on exhibition: Holland Organizing Centre, 16 Lange Voorhout, The Hague, The Netherlands

A NEW MEETING IS ANNOUNCED BY HOLLAND ORGANIZING CENTRE

Name: International Symposium on Fluorescein Angiography
Type: Medical Symposium
Date: 28 March—1 April, 1976
Place: Ghent, Belgium
Building: not yet known
Organizing body and/or sponsor: —
Secretariat: c/o Holland Organizing Centre, 16 Lange Voorhout, The Hague, The Netherlands
Subject: Fluorescein Angiography
Languages used: English, French
Simultaneous interpretation: yes
Attendance (est.): 300
Free (Limited) On invitation only
Number of papers: ca. 120
Free (Limited) On invitation only
Language of papers: English, French
Deadline for abstracts: 1 December, 1975
Deadline for complete texts: Congress date
Abstracts will be published in: book form
Date of publication: March 1976
Full papers will be published in: book form
Date: December 1976
Price: ±Dfl. 100,—
Obtainable from: Dr. W. Junk N. V. — Publishers, 13 van Stolkweg, The Hague, The Netherlands
Exhibition: yes
Number of exhibitors (est.): 20
Subject: technical and scientific; medical equipment, pharmaceuticals
Information on exhibition: Holland Organizing Centre, 16 Lage Voorhout, The Hague, The Netherlands

A NEW MEETING IS ANNOUNCED BY HOLLAND ORGANIZING CENTRE

Name: 5th Congress of the European Society of Ophthalmology
Type: Scientific Medical Congress
Date: 5—9 April, 1976
Place: Hamburg, German Federal Republic
Building: Congress Centre Hamburg
Organizing body and/or sponsor: The German Society of Ophthalmology and the Professional Association of German Ophthalmologists
Secretariat: c/o Holland Organizing Centre, 16, Lange Voorhout, The Hague, The Netherlands
Subject: Vascularization of the Uvea, the Retina and the Optic Nerve; Anatomy and Pathology
Languages used: English, French, German
Simultaneous interpretation: yes
Attendance (est.): 1500
Free
Number of papers: 130 + 20
Free (Invited)
Language of papers: English, French, German
Deadline for abstracts: 1 October, 1975
Deadline for complete texts: 1 April, 1976
Abstracts will be published in: book form
Date of publication: 1 April, 1976
Full papers will be published in: book form
Date: not yet available
Price: not yet available
Obtainable from: not yet available
Exhibition: yes
Number of exhibitors (est.): 75
Subject: Technical and scientific; medical equipment, pharmaceuticals
Information on exhibition: Holland Organizing Centre, 16, Lange Voorhout, The Hague, The Netherlands

INTERNATIONAL SYMPOSIUM ON FLUORESCEIN ANGIOGRAPHY ISFA

Ghent, 28 March—1 April, 1976

Announcement

The next International Symposium on Fluorescein Angiography — ISFA — will be held at Ghent, Belgium, from 28 March—1 April, 1976

Organisation Committee

Chairman: J. François
Secretary: J. J. De Laey
Members: P. Amalric, A. Bird, A. Deutman, J. Oosterhuis, E. Norton, A. Wessing, K. Shimizu

Scientific Programme

The main topics will be fluorescein angiography of pigment-epithelium, choroid and retinal periphery. Sessions will, however, also be devoted to instrumentation and techniques, ocular hemodynamics (including retinal vein thrombosis) and diabetes. Each session will be introduced by invited lectures. In order to reserve ample time for discussion, the number of free papers will be limited.

Please notify the Secretariat, by means of the enclosed form, of your intention to submit a paper. All relevant details will then be forwarded.

Languages

The official languages of the Symposium are English and French. Simultaneous interpretation in these languages will be provided.

Exhibition

During the Symposium both a scientific and a commercial exhibition will be held.

Those interested in participating in one of these exhibitions, are requested to apply to the Secretariat by means of the enclosed form. They will then receive all relevant information.

Social Programme

An informal gathering will be held on Sunday, 28 March, after the Opening Session, while also a general excursion, followed by a farewell party, is included in the programme.

For the accompanying persons a special programme, including a full day's excursion to Brussels, is being arranged.

Transportation

Sabena (Belgian World Airlines) has been appointed official carrier for the Congress. For all information concerning your air-transportation to Ghent, please apply to the nearest Sabena office.

Participation

If you are interested in participating in this Symposium, you are invited to forward your name and address to the Secretariat by means of the enclosed form. In due course you will then receive the Provisional Programme and official application form.

A NEW MEETING IS ANNOUNCED BY HOLLAND ORGANIZING CENTRE

Name: 7th European Congress of Cardiology
Type: International Scientific Medical Congress
Date: 20—25 June, 1976
Place: Amsterdam
Building: Congress Centre RAI
Organizing body and/or sponsor: The Netherlands Society of Cardiology
Secretariat: c/o Holland Organizing Centre, 16, Lange Voorhout, The Hague, The Netherlands
Subject: Coronary Artery Disease; Drug Therapy; Ventricular Functions; Myocardiopathies
Languages used: English, French, German
Simultaneous interpretation: yes
Attendance (est.): 3000
Free
Number of papers: ±50 invited papers
Free (Invited)
Language of papers: English, French
Deadline of abstracts: 1 January, 1976
Deadline for complete texts: 15 June, 1976
Abstracts will be published in: book form
Date of publication: 15 June, 1976
Full papers will be published in: not yet known
Date: not yet known
Price: not yet known
Obtainable from: not yet known
Exhibition: yes
Number of exhibitors (est.): ca. 150
Subject: scientific, and technical; medical equipment, pharmaceuticals
Information on exhibition: Holland Organizing Centre, 16, Lange Voorhout. The Hague, The Netherlands

VIth INTERNATIONAL CONGRESS OF SOCIAL PSYCHIATRY

OCTOBER, 1976

In October, 1976 the VIth International Congress of Social Psychiatry will be held in Yugoslavia, but it has not yet been decided in which town.

Colleagues, neuropsychiatrists, specialists in the other fields of medicine, general practitioners, psychologists, defectologists, music therapists, sociologists, social workers, nurses, and other specialists in medical and non-medical professions who are interested to participate in the Congress, are pleased to write for more detailed information on the Congress, to the president or the secretary of the International Association of Social Psychiatry:

General Secretary:
JOHN J., CARLETON, M. D.
American Association for Social Psychiatry
The Santa Barbara Psychiatric Medical Group
2323 Oak Lane
Santa Barbara, Calif. 93105 U.S.A.

President:
Prof. Dr. VLADIMIR HUDOLIN
University Department for Neurology and Psychiatry of Dr. M. Stojanović University Hospital, Vinogradska c. 20
41000 Zagreb, Yugoslavia

A NEW MEETING IS ANNOUNCED BY HOLLAND ORGANIZING CENTRE

Name: 5th International Conference on Birth Defects
Type: Conference on Birth Defects
Date: 21—27 August, 1977
Place: Montreal
Building: Queen Elizabeth Hotel
Organizing body and/or sponsor: National Foundation — March of Dimes
Secretariat: c/o Holland Organizing Centre, 16, Lange Voorhout, The Hague, The Netherlands
Subject: Birth Defects
Languages used: English, French
Simultaeneous interpretation: English, French
Attendance (est): 900
Free
Number of papers: ca. 130
Free (Invited)
Language of papers: English, French
Deadline of abstracts: not yet known
Deadline for complete texts: not yet known
Abstracts will be published in: book form (English)
Date of publication: August 1977
Full papers will be published in: book form (English)
Date: Summer 1978
Price: not yet known
Obtainable from: Excerpta Medica Foundation, Jan van Galenstraat 335, Amsterdam, the Netherlands
Exhibition: no
Number of exhibitors (est.): —
Subject: —
Information on exhibition: —

The Excerpta Medica Foundation announces the convening of the

First international congress on patient counselling

which will be held from 21st-23rd April 1976
in Amsterdam, The Netherlands

PLENARY SESSIONS will deal with the following subjects:

- Should the patient be told the truth?
- Doctor awareness of patient counselling needs
- Techniques of patient counselling
- Legal aspects of being a patient
- Patient counselling in psychiatric illness
- Labelling of drugs
- Influence of the mass media on patient behaviour
- Patient counselling as the beginning of social action

SECTIONAL SESSIONS will be devoted to the following topics:

- Patient counselling in hospital treatment
- Patient counselling in chronic diseases
- Death and dying
- Communicating with the mentally retarded
- Adjustment to loss of major body function
- Patient counselling and the general practitioner
- Patient counselling as a part of medical training
- The role of the health professional in patient counselling
- Patient counselling in paediatrics
- Patient counselling in geriatrics

The Excerpta Medica Audiovisual Patient Information Award

The Excerpta Medica Foundation will award a prize for the best audivosual programme dealing with patient counselling submitted for display at the Congress.

FOR FURTHER DETAILS:

First International Congress on Patient Counselling
c/o Excerpta Medica Foundation
P.O. Box 1126
Amsterdam, The Netherlands

VII.

INTERNATIONAL SYMPOSIUM OF THE
DEUTSCHE AKADEMIE FÜR PSYCHOANALYSE (DAP) e.V.

Congress Center of the San Domenico Palace Hotel
I-98039 Taormina/Sicily (Italia) August 1st to 5th, 1975

"PSYCHOANALYTIC TRAINING"

Chairmanship: Günter Ammon, M. D.
Organization Committee: D-1 Berlin 15, Wielandstrasse 27/28
Registration: August 1st, 1975, from 6.00 p. m.
Opening: August 2nd, 1975, 10.00 a.m., in the Plenary Hall of the San Domenico Palace Holtel

Papers and Short Papers concerning the following subjects which are also the subjects of the workshops taking place simultaneously can be submitted until the 1st of April 1975 (Synopses must be presented till February 1st, 1975):

1. Theory and Practice of the Psychoanalytic Training
2. Training Analysis
3. Individual and Group Control Analysis
4. Training in Analytic Group Therapy, Group Dynamics and Milieu Therapy
5. Psychodynamics of a Training Institute
6. Relation between Training and Research within Psychoanalytic Institutes: Pros and Cons
7. Relation between Psychoanalysis and Psychiatry during the Training
8. Relation between Psychoanalytic Institutes and University Institutes
9. The Identity of the Psychoanalist in Society

Furthermore there will be a Panel-Discussion:

"THEORY AND PRACTICE OF PSYCHOANALYTIC TRAINING"

Registration Fee: If registered till May 31st, 1975: DM 300,— from June 1st, 1975 DM 350,—

Information concerning Participation Papers, Workshops etc. may be obtained from the Organization Committee as well as from the

Lehr- und Forschungsinstitut für Dynamische Psychiatrie und Gruppendynamik (LFI)
1 Berlin 15
Wielandstraße 27 – 28
Telefon 030/883 92 24

Münchener Lehr- und Forschungsinstitut der DAP
8 München 40
Leopoldstraße 87
Telefon 089/34 14 44

Düsseldorfer Lehr- und Forschungsinstitut der DAP
4 Düsseldorf
Schadowstraße 86 – 88
Telefon 0211/36 49 00

Frankfurter Lehr- und Forschungsinstitut der DAP
6 Frankfurt 1
Niedenau 36
Telefon 0611/72 53 41

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Printed in Hungary

A kiadásért felel az Akadémiai Kiadó igazgatója. **Műszaki szerkesztő: Zacsik Annamária**

A kézirat nyomdába érkezett: 1975. VII. 2. — Terjedelem: 16,80 (A/5) ív, 58 ábra

75.2017 Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György

ACTA MEDICA

ТОМ. 31 – ВЫП. 3–4

РЕЗЮМЕ

ГЕМОДИНАМИЧЕСКИЕ ИЗМЕНЕНИЯ, НАБЛЮДАЕМЫЕ У СОБАКИ ПОД ВЛИЯНИЕМ НОРАДРЕНАЛИНА И ИЗОДИГИДРОПЕРПАРИНА

Г. ПОГАЧА, Е. ДУБЕЦ и Д. ГАБОР

Авторы выявили, что у собаки после одновременного введения норадреналина и изодигидроперпарина возникновение застоя лимфы в тканях и отечной инфильтрации сосудистой стенки сопровождается гипертонией, повышением центрального венозного давления и ускорением тока лимфы.

ВЛИЯНИЕ ФРУКТОЗЫ НА ОБМЕН МОЧЕВОЙ КИСЛОТЫ

Э. ХОЛЛЕНДЕР

После вливания фруктозы в плазме и моче здоровых людей и больных подагрой наблюдается повышение содержания мочевой кислоты. Однако, в то время как здоровые почки быстро выделяют избыток мочевой кислоты, в плазме больных подагрой уровень мочевой кислоты еще четыре часа после введения фруктозы остается повышенным.

В результате введения крысам оксониевой кислоты, ингибирующей уриказу, устанавливаются подобные изменения содержания мочевой кислоты в плазме как и у больных подагрой. Возникающую под влиянием фруктозы гиперурикемию можно объяснить промежуточными изменениями обмена веществ, а не повышением диуреза и сочетающейся дегидратацией, вызванными дачей фруктозы.

ВЛИЯНИЕ ПРОСТАГЛАНДИНОВ И ПРЕПАРАТА НО-ШПА (СОЛЯНОКИСЛОГО ДРОТАВЕРИНА) НА АГРЕГАЦИЮ ТРОМБОЦИТОВ, ВЫЗВАННУЮ АДФ

Х. ЛОШОНЦИ, И. НАДЬ, З. ГРЕГУШ

Обсуждается действие простагландинов E_1 и E_2 (фирмы Хиноин) на агрегацию тромбоцитов in vitro. В соответствии с литературными данными $ПГЕ_1$ оказался очень эффективным, а $ПГЕ_2$ несколько менее энергичным ингибитором агрегации. Ингибиторное действие НО-ШПА (фирмы Хиноин), известного спазмолитика, на агрегацию кровяных пластинок, вызванную АДФ, авторами было выявлено уже в прежней работе. Ввиду того, что задерживающее агрегацию действие НО-ШПА проявляется только при большой концентрации, авторы изучали совместное действие НО-ШПА и $ПГЕ_1$, а также НО-ШПА и $ПГЕ_2$. В результате потенцирующего синергизма, комбинация этих двух препаратов in vitro вызывает полную задержку агрегации уже в таких концентрациях, которые при изолированном введении НО-ШПА не влияют, или почти не влияют на агрегацию. Обсуждается ожидаемое значение этого наблюдения в профилактике тромбоза.

ОБОСОБЛЕНИЕ ОТДЕЛЬНЫХ ФРАКЦИЙ КРОВЯНЫХ КЛЕТОК И ТЕСНАЯ СВЯЗЬ МЕЖДУ ЭНДОДЕРМАЛЬНЫМ ЭПИТЕЛИЕМ И ГЕМОПОЭТИЧЕСКИМИ ПРЕКУРСОРАМИ В ЧЕЛОВЕЧЕСКОМ ЖЕЛТОЧНОМ МЕШКЕ

Э. КЕЛЕМЕН

При микроскопическом изучении человеческого желточного мешка автор наблюдал определенное обособление гемопоэтических клеток: ранние прекурсоры гемопоэза, не содержащие гемоглобин, располагались вблизи эндодермы, тогда как эмбриональные эритробласты с богатой эозинофильными клетками плазмой были расположены прежде всего в сосудах или непосредственно вокруг последних.

Гемопоэтические прекурсоры иногда находятся в эндодермальном клеточном мешке. Хотя еще не имеются доказательства об эндодермальном происхождении прекурсоров гемопоэза или о проникновении, по-видимому замкнутых, прекурсоров в кровообращение, тесная связь между эпителием эндодермы человеческого желточного мешка и прекурсорами гемопоэза требует объяснения.

АВТОИММУННОЕ СОСТОЯНИЕ БОЛЬНЫХ БОЛЕЗНЬЮ БАЗЕДОВ-ГРЕВСА

И. ЭРДЕИ, Ш. ФАЗЕКАШ, Б. КИШ, Д. СЕГЕДИ и Д. ПЕТРАНЬИ

На основе клинического и лабораторного обследования 175 больных болезнью Базедов-Гревса, лечившихся предварительно радиоактивным иодом, 17 больных, не получивших еще лечения, и лабораторного исследования 44 здоровых лиц было установлено, что

1. с болезнью Базедов-Гревса сочетаются многочисленные (прочие) автоиммунные заболевания;

2. у больных болезнью Базедов-Гревса титры противощитовидных, противомышечных и противопочечных антител достоверно превышают титры контрольных лиц. В случае антинуклеарной реакции наблюдается подобное положение;

3. степень повышения титров противотел в случае противощитовидных антител и антинуклеарной реакции показывает достоверную корреляцию со временем, протекшим после начала заболевания. В течение 7—8 лет после начала заболевания титры повышаются, а начиная с 15-го года они достоверно понижаются.

СРАВНИТЕЛЬНЫЕ ВНУТРИСЕРДЕЧНЫЕ И МЕХАНОГРАФИЧЕСКИЕ ИССЛЕДОВАНИЯ В ОПЫТАХ НА ЖИВОТНЫХ

Л. ВОЙТ и Л. МИХОЦИ

Авторы изучали в опытах на животных полученные кровавым путем (кривые давления в правом и левом желудочках, или в аорте и легочной артерии) и бескровным путем (правую и левую верхушечную кардиограмму) данные о различных фазах сердечного цикла, прежде всего относительно периода перед выбрасыванием крови и времени выбрасывания.

Согласно результатам:

1. данные, полученные кровавым и бескровным путем, не показывают значительных отклонений;

2. верхушечная кардиограмма начинается раньше, чем соответствующая кривая давления в желудочках;

3. временный ход правой и левой верхушечной кардиограмм неодинаков;

4. в опытах на животных точка *E* верхушечной кардиограммы кажется подходящей для определения начала выбрасывания крови.

ГЛОМЕРУЛОНЕФРИТ ИММУННОКОМПЛЕКСНОГО ПРОИСХОЖДЕНИЯ, СОПРЯЖЕННЫЙ БОЛЕЗНЬЮ ХОДЖКИНА

Й. САБО, Д. ЛУШТЬИК, И. ЭРДЕИ и Д. СЕГЕДИ

Сообщается случай больного болезнью Ходжкина, у которого развился нефротический синдром. Микроскопическое и электроннооптическое исследование биоптического материала почек показало мембранопролиферативный гломерулонефрит иммунокомплексного происхождения. Связь между нефротическим синдромом и протеканием болезни Ходжкина указывает — в соответствии с литературными данными — на этиологическую связь двух заболеваний.

ТРОМБЭЛАСТОГРАФИЧЕСКОЕ ИССЛЕДОВАНИЕ БОЛЬНЫХ, ПРЕТЕРПЕВШИХ СПЛЕНЕКТОМИЮ

М. МИС, Б. ШИРО и Б. ШАРИ

Авторы изучали тромбэластограммы 28 лиц, претерпевших спленектомию по разным причинам. Как в группе лиц, у которых селезенку удалили по поводу травмирования (значит у, по существу, здоровых лиц), так и в группе больных, оперированных по поводу заболевания печени, наблюдались отклонения ТЭГ, указывающие на склонность к повышенной свертываемости. Авторы настоящей статьи не наблюдали явления ступеней, описанного другими исследователями.

«КВАНТИФИКАЦИЯ ПРОСТРАНСТВЕННЫХ РАЗМЕРОВ И ПРОСТРАНСТВЕННОЙ СКОРОСТИ НОРМАЛЬНОГО И ПАТОЛОГИЧЕСКОГО QRS КОМПЛЕКСА»

АНТАЛОЦИ, З., ШТРОММЕР, М., РЕГЕШ, Л.

В четырехстах сорока одном случае исследованы пространственные размеры и пространственная скорость нормального и патологического QRS комплекса. Пространственные размеры определены с помощью триаксикардиометрической аналоговой вычислительной машины, пространственная скорость вычислена с помощью вычислительной машины. Аналогоны пространственных размеров (M), азимута (H°) и элевации (V°) дигитилизованы при разбивке на мгновенные 10 мс векторы и числовые данные компонентов XYZ получены при использовании угловых функций на электронной вычислительной машине ОДРА 1204. По данным компонентов на основе формулы пространственной скорости (SV) вычислены скалярные значения пространственной скорости. На основе числовых данных, действительных для пространственных размеров и пространственной скорости по 10 мс циклам и, соответственно, 1/8 циклам, графически построены кривые пространственных размеров и пространственной скорости и анализированы их средние значения по группам больных (а именно графические алгоритмы).

ИММУНОЛОГИЧЕСКИЕ ОТНОШЕНИЯ ПРИ ХРОНИЧЕСКОМ ПИЕЛОНЕФРИТЕ КЛЕТОЧНЫЙ ИММУННЫЙ ОТВЕТ ПРИ ХРОНИЧЕСКОМ ПИЕЛОНЕФРИТЕ

Т. САБО, Б. ФЕКЕТЕ и Д. ПЕТРАНЬИ

Авторы изучали при помощи пробы на образование розетки иммунологическую сенсибилизацию, развивающуюся при инфекции в ходе хронического пиелонефрита (ХПН), так как в случае наличия специфических антигенов число клеток, образующих розетки, дает ориентировку о степени иммунного ответа, вызванного антигенами. Было найдено, что в период обострения процесса ХПН-а, в случае заражения кишечной палочкой, в преобладающем большинстве случаев получается положительная проба на образование розетки. В случае же инфекции синегнойной палочкой и протеем при исследовании О-антигенов последних не удалось получить положительного ответа. В связи с инфекци-

ями нижних мочевых путей был получен иммунный ответ меньшего размера. Из этих результатов можно заключить, что более выраженный иммунный ответ получается только в случае инфекций, распространяющихся также на паренхиму почек, причем эта реакция является только временной и не защищает больных от рецидивов и реинфекций. Важнейшей целью и в дальнейшем является предотвращение поселения микроорганизмов и их антигенов на месте иммунологических событий, в интерстиции почки.

ПРОЯВЛЕНИЕ НАСЛЕДСТВЕННЫХ ЗАБОЛЕВАНИЙ ПЕЧЕНИ ВСЛЕДСТВИЕ МЕДИКАМЕНТОЗНОГО ЛЕЧЕНИЯ

Т. ХОРВАТ, А. ГОГЛ, Ч. РУЖА, А. ЛУДАНЬ и Т. ЯВОР

Сообщаются 4 наблюдения, при которых под влиянием медикаментозного лечения до тех пор неизвестная энзимопатия стала явной. На основе наблюдений, сделанных у двух больных с болезнью Жильбера и у двух больных с острой интермиттирующей порифрией, обсуждается значение подверженности генетической опасности, проявляющейся подвлиянием медикаментозного лечения.

ЭФФЕКТ ОСТРОГО ХОЛЕСТАЗА НА КРОВООБРАЩЕНИЕ ПЕЧЕНИ

Г. САБО, Ф. ЯКАБ и З. МАДЬЯР

Авторы изучали у наркотизированных собак при помощи электромагнитного амперметра кровоток в печеночной артерии и в воротной вене до закрытия и в течение 6 часов после закрытия желчного протока. Параллельно повышению давления в желчных путях в печеночной артерии повышается кровоток и понижается сопротивление, в то время как в воротной вене сопротивление увеличивается и кровоток падает. Полный кровоток печени в сущности остается неизменным. Анализ результатов по-видимому указывает на то, что между изменениями кровотока и в печеночной артерии и в воротной вене существует более тесная связь, чем между повышением давления в желчных путях и кровотоком в отдельных сосудах. Эти наблюдения можно согласовать с предположением, по которому повышение кровотока в печеночной артерии имеет первичное значение в изменении кровотока печени. Повышенное вследствие холестаза тканевое давление посредством снижения чрезстеночного давления вызывает в печени миогенное расширение артериол. Между реципрокным сопротивлением в печеночной артерии и сопротивлением в воротной вене существует обратная линейная корреляция. Понижение портального кровотока обусловливается, главным образом, повышением предпеченочного брыжеечного сопротивления. Последнее вызывается повышением портального сопротивления. Между портальным и брыжеечным сопротивлениями можно выявить экспоненциальную связь.

МЕХАНИЗМ ИЗМЕНЕНИЙ КРОВОТОКА В ПЕЧЕНИ, ВЫЗВАННЫХ ПОВЫШЕНИЕМ ДАВЛЕНИЯ В ЖЕЛЧНЫХ ПУТЯХ

Д. САБО, Ф. ЯКАБ, Ж. МАДЬЯР

У собаки внезапное повышение давления в желчных путях до 40 мм рт. ст. значительно (+39%) повышает кровоток в печеночной артерии, в то время как в воротной вене кровоток понижается только в умеренной мере (−9,5%). При постепенном повышении давления желчи кровоток печеночной автории вначале повышается только умеренно, а затем, при достижении критической величины между 20—35 мм рт. ст., наступает внезапное повышение почти до максимума. Блокада адренергных α- и -β-рецепторов и дача атропина не влияют на эту реакцию. Изменение состава жидкости, наполняющей желчные пути, не оказывает влияния на размер вазодилятации, но изменяет протекание процесса. Указанное явление можно объяснить миогенным расширением артериол, вызванным понижением эффективного трансстеночного давления артериол.

ОСЛОЖНЕНИЯ ПРИ ИСТИННОЙ КРАСНОЙ ПОЛИЦИТЕМИИ И ЕЕ СОПРЯЖЕНИЕ С ДРУГИМИ НЕГЕМОЛИТИЧЕСКИМИ ЗАБОЛЕВАНИЯМИ

Д. НАДЬ

На основе данных 156 больных истинной красной полицитемией, наблюдавшихся в течение 14 лет, автор сообщает частоту встречаемости сосудистых осложнений, а также частоту сочетания этой болезни с другими заболеваниями.

В больничном материале автора до начала активного лечения и систематической гематологической диспансеризации у 48 больных появились-какие нибудь сосудистые осложнения. В связи с болезнью Вакез—Ослера часто наблюдались гипертония, декомпенсация сердца, грудная жаба, сахарный диабет или диабетоидная кривая сахара крови и язвенная болезнь.

Patogenez oshogowoi anemii

PATHOGENESE DER VERBINDUNGSANÄMIE

VON I. BERNÁT

In dieser Monographie wird erstmalig eine zusammenfassende Darstellung der im Verlauf der Verbrennungskrankheit auftretenden und zur Anämie führenden Stoffwechselstörungen gegeben. Außer eingehender Abhandlung der Forschungsergebnisse des Autors auf dem Gebiet des Eisenstoffwechsels und der Häm-, bzw. Globin-Synthese, sind weitere Abschnitte der Monographie dem Typ und dem Verlauf der Verbrennungsanämie, den morphologischen, biochemischen und funktionellen Eigenschaften bzw. der Lebensdauerbestimmung der sich nach einer thermischen Verletzung bildenden Erythrozyten gewidmet.

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Iconographia selecta dermatohistologica

EIN DERMATOHISTOLOGISCHER ATLAS

VON L. SZODORAY und K. VEZEKÉNYI

Vermöge des überaus reichen Bildmaterials (415 größtenteils Mikrofilme und klinische Aufnahmen in schwarzweiß) dürfte dieses Werk in der einschlägigen Literatur der letzten Jahre einen ganz besonderen Platz einnehmen. Zu einer besseren Information des Lesers dienen mehrere Übersichtstafeln der Krankheitsgruppen (Mechanismus der Blasenbildung usw.), sowie in einigen Abschnitten die Darstellung der histochemischen Ergebnisse.

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AKADÉMIAI KIADÓ
BUDAPEST

WIRKUNGSMECHANISMUS SYNTHETISCHER PROGESTOGENE

von F. SZONTÁGH

Das Buch behandelt ein hochaktuelles Thema: der Wirkungsmechanismus der Progestogene – dieser reproduktionsmäßig sehr aktiven Wirkstoffgruppe – wird experimentell und klinisch vielseitig untersucht. Der Einleitung folgt die Beschreibung und statistische Auswertung der angewandten Methoden. Es ergibt sich die Schlußfolgerung, daß der Mechanismus der empfängnisverhütenden Wirkung weitaus komplizierter ist, als bisher angenommen wurde. Beim Zustandekommen dieser Wirkung spielen mindestens 4, wahrscheinlich aber 6 Angriffspunkte eine Rolle. Der Verfasser bewertete die einzelnen Angriffspunkte auch in der Hinsicht, ob ein Wirkungsmechanismus allein – und wenn, so welcher – den antikonzeptionellen Effekt erzeugen kann. Anschließend werden die Untersuchungen über die therapeutische Anwendung der Progestogene zusammengefaßt. Als Abschluß bietet das Buch eine kurze Darstellung der Studien, die für eine trophoblast-aktivierende Wirkung einiger Progestogene sprechen.

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COR PULMONALE CHRONICUM

VON I. SZÁM

Definition, Geschichte, Häufigkeit, Pathologie, Formen, Ursachen, Entstehungsmechanismus, Hämodynamik des cor pulmonale chronicum werden ausführlich dargelegt. Das Buch behandelt in diesem Zusammenhang auch die von der pulmonalen Hypertonie verursachten morphologischen Herzmuskelveränderungen, die Respirationsstörung. Das chronische cor pulmonale ist nicht nur eine Krankheit der Herzens, sondern des ganzen Organismus; in diesem Sinne wurde das Kapital über die Veränderungen der einzelnen Organe (Gehirn, Leber, Niere, Lunge, blutbildende Organe) ausgearbeitet; besonders eingehend ist die Beschreibung der Schädigungen des Zentralnervensystems. Nach einer gründlichen Erörterung der Symptomatologie folgt die kritische Auswertung der diagnostischen Methoden sowie die sich auf alles erstreckende Auseinandersetzung der therapeutischen Möglichkeiten. Das letzte Kapitel behandelt die Rehabilitationsaussichten, die Prognose und Prophylaxe der Krankheit und die in dem letzten Jahrzehnt verzeichneten Änderungen des klinischen Krankheitsbildes. Tabellen und eine Bibliographie vervollständigen das Werk.

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