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PARTIELLE DISSOZIATION DER RECHTEN KAMMER (MECHANISCHE ATRIALISATION) BEI EBSTEINSCHER ANOMALIE

Von

K. LOZSÁDI und K. SÁRKÖZY

CHIRURGISCHE ABTEILUNG (CHEFARZT: DR. A. TEMESVÁRI) DES INSTITUTS FÜR KARDIOLOGIE, BUDAPEST

(DIREKTOR: PROF. G. GOTTSEGEN †)

(Eingegangen am 3. März 1966)

Bei einem an EBSTEINscher Anomalie leidenden Patienten wurde intraoperativ die funktionelle Atrialisation des anatomisch atrialisierten proximalen Kammerabschnitts beobachtet; der erwähnte Kammerabschnitt verrichtete mit den Vorhöfen synchrone Vorhofkontraktionen. Der durch die Rhythmusveränderung herbeigeführte systemische Blutdruckabfall konnte lediglich mittels Noradrenalininfusion normalisert werden.

Es wird versucht, die beobachteten Erscheinungen mit Hilfe der in der Literatur mitgeteilten indirekten Angaben zu erklären.

In der Gruppe der kongenitalen Vitien beträgt die Häufigkeit der EBSTEINschen Anomalie [1] weniger als 1% [2]. Der Umstand, daß die elektrokardiographischen und hämodynamischen Untersuchungen fast in jedem Fall überraschende pathologische Raritäten an den Tag bringen, kann wahrscheinlich dem seltenen Vorkommen zugeschrieben werden.

Der anatomische Charakter der Anomalie bringt im rechten Herzen eigenartige Kreislaufverhältnisse zustande. Da 1 oder 2 Segel der Trikuspidalklappe ihren Ursprung unterhalb des Annulus auf dem Endokard der rechten Kammer haben, gliedert sich das rechte Herz anatomisch als auch funktionell in 3 Teile. Die Klappen anomalen Ursprungs teilen die rechte Kammer in einen proximalen und einen distalen Abschnitt (Abb. 1: R. K. $_{I.}$ und R. K. $_{II.}$) Der dünnwandige distale Abschnitt gehört nur funktionell zur rechten Kammer und ist mit dem rechten Vorhof zusammengeschmolzen, d. h. atrialisiert [3, 4]. Der Vorhof besteht demzufolge eigentlich aus 2 Höhlen: Ein Teil des Vorhofs, der eigentliche rechte Vorhof verrichtet die Vorhofkontraktion, während die Aufgabe der Kammerkontraktionen dem proximalen Kammerabschnitt zufällt. Diese letzterwähnten Kontraktionen sind jedoch im Verhältnis zu den Kontraktionen des dickwandigeren distalen Kammerabschnitts träger.

Die anatomische und funktionelle Paradoxie des proximalen rechten Kammerabschnitts kann auch weitere Konsequenzen zur Folge haben. Wie das auch aus unserem Fall hervorgeht, kann der atrialisierte Kammerabschnitt nicht nur im Kammerrhythmus sondern auch im Vorhofrhythmus, synchron mit dem rechten Vorhof funktionieren.

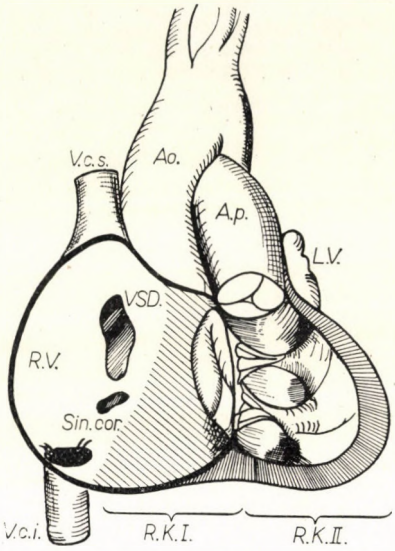

Abb. 1. Schematische Darstellung der EBSTEINschen Anomalie. Simultane Kontraktion des rechten proximalen (R. $K._{I.}$) und distalen (R. $K._{II.}$) Kammerabschnitts. Während der Dissoziation synchrone Kontraktion des proximalen Abschnitts (schraffiertes Gebiet) und des rechten Vorhofs (R. V.).V.c.s. = V. cava sup., V.c.i. = V. cava inf., Sin. cor. = Sinus coronarius, VSD = Vorhofseptumdefekt, A. p. = A. pulmonalis, L. V. = Linker Vorhof, Ao. = Aorta

Falldarstellung

L. D. 10jähriger, körperlich und geistig mangelhaft entwickelter Knabe. An Gesicht, Lippen und Nägeln geringe, bei Belastung stärker werdende Zyanose. Trommelschlägerfinger geringen Grades. Rachitischer Brustkorb. Negativer Lungenbefund. Die Herzgrenzen überschreiten rechts das Sternum, links die medioklavikuläre Linie; Spitzenstoß im V. Zwischenrippenraum. Reine normale Herztöne. Links parasternal protosystolisches Geräusch, im linken III.—IV. Interkostalraum von der Protosystole ausgehendes kurzes Geräusch. Gespalteter, akzentuierter II. Ton. Herzfrequenz 96/min, rhythmisch. Leber und Milz nicht palpierbar. Blutdruck 110/80 mmHg, Erythrozytenzahl 4 000 000, Hb 88%. EKG: rechter Schenkelblock, in Ableitung V_1 hohe P-Wellen, in Ableitungen V_{1-3} auf Überbelastung des rechten Herzens weisende Zeichen. PKG ergibt außer dem Auskultationsbefund Füllungs- und Vorhoftöne. Thoraxröntgenbefund: normale Hili, das Herz ist in beiden Richtungen breiter; flacher Herzsinus. Auf den Schrägaufnahmen sind die wesentlich erweiterte rechte und die mäßig dilatierte linke Kammer sichtbar. Beide Vorhöfe sind vergrößert. Elongierte, erweiterte Aorta. Bei der Rechtsherzkatheterisierung normale Druckwerte. Der Katheter gelangte vom rechten in den linken Vorhof und von dort in die linke Kammer. Aus den 2 letzterwähnten Stellen waren desaturierte Blutsauerstoffwerte zu gewinnen. Angiokardiogramm des rechten Vorhofs: erweiterter rechter Vorhof, nicht größere rechte Kammer, grazilere Pulmonalarterien. Dextrogramm: keine Füllung des linken Vorhofs, der linken Kammer bzw. der Aorta. Lävogramm: die Höhlen sind von Normalgröße, die Wiederauffüllung des rechten Herzens ist nicht sichtbar.

In Anbetracht, daß das geeignete Operationsverfahren (Verschließung des Vorhofdefektes, V. cava-pulmonalis-Anastomose) nur anhand der intrakardialen Befunde festgestellt werden konnte, wurde in 30 °C Hypothermie die Thorakotomie vorgenommen. Wesentlich vergrößerter rechter Vorhof: mit der Ausströmungsbahn synchrone Kontraktion der stark dilatierten dünnwandigen Einströmungsbahn der rechten Kammer. Zwischen dem proximalen und distalen Kammerabschnitt ist eine geringe Einziehung ersichtlich. Um die Größe des Vorhofseptumdefekts und Lokalisation der Segel der Trikuspidalklappe feststellen zu können,

wurde durch das rechte Aurikel digitale Exploration durchgeführt. Am Vorhofseptum ließ sich ein etwa fingerbeergroßer Defekt »secundum« Typs identifizieren. Der Vorhof setzte sich in Richtung der Kammerhöhle fort, die dem Annulus entsprechende ausgeprägte atrioventrikuläre Grenze war nicht palpierbar. Lediglich das septale Segel der Trikuspidalklappe hatte seinen Ursprung in Normalhöhe, das andere Klappensegel war in der Höhe der Ausgangsstelle der Ausströmungsbahn jenem Sulcus entsprechend, welcher die rechte Kammer in 2 Teile teilt, zu palpieren. Die Richtung der Klappenebene war fast senkrecht. Nach eindeutiger Klärung der EBSTEINschen Anomalie und des damit verbundenen Vorhofseptumdefektes wurde die Exploration beendet und die Druckwerte der Herzhöhlen bestimmt.

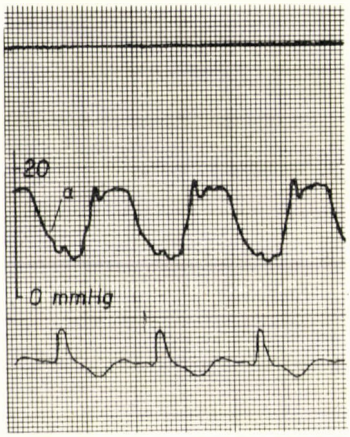

Abb. 2. Intraoperative Druckkurve der rechten Vorhof-proximalen rechten Kammer-Höhle während der simultanen Depolarisation des proximalen und distalen rechten Kammerabschnitts

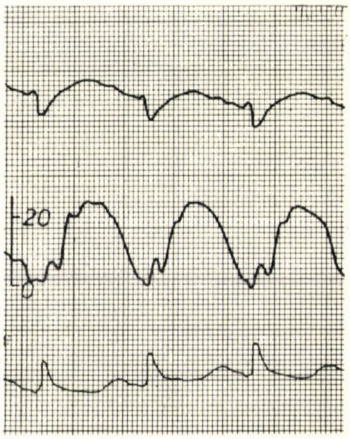

Abb. 3. Intraoperative Druckkurve des distalen rechten Kammerabschnitts während der simultanen Depolarisation des proximalen und distalen rechten Kammerabschnitts

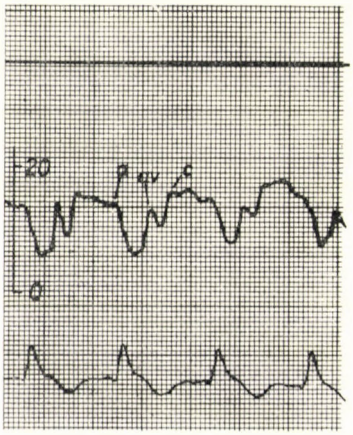

Abb. 4. Intraoperative Druckkurve des rechten Vorhofs

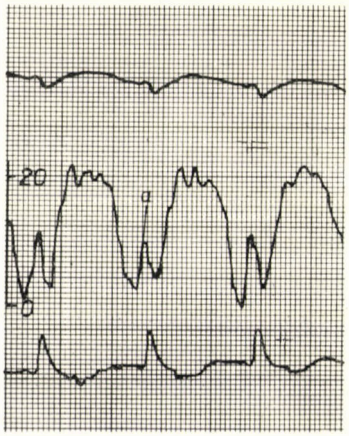

Abb. 5. Intraoperative Druckkurve der rechten Vorhof-proximalen rechten Kammer-Höhle während der simultanen Depolarisation des rechten Vorhof-proximalen Kammerabschnitt-Komplexes

Intraoperativ gemessene intrakardiale Druckwerte. Druck in der gemeinsamen Höhle des rechten Vorhofs und des proximalen Kammerabschnitts: 17/5 mmHg (Abb. 2), im distalen Teil der rechten Kammer: 25/0 mmHg (Abb. 3), der A. pulmonalis: 22/14 mmHg, des linken Vorhofs: 16/6 mmHg (Abb. 4). Vor dem Beginn der eigentlichen Operation änderte die proximale rechte Kammer plötzlich ihren Rhythmus und verrichtete anstatt den bisherigen Kammerkontraktionen mit dem rechten Vorhof synchrone Kontraktionen. Die Vorhofsystole lief über die Einströmungsbahn der rechten Kammer bis zur Ausströmungsgrenze, die darauffolgende Kammersystole war lediglich an der Ausströmungsbahn ersichtlich. Gleichzeitig verminderte sich der systemische Blutdruck von 80 mmHg auf 40 mmHg. Auf der Druckkurve des sich synchron kontrahierenden rechten Vorhof-proximalen rechten Kammer-Komplexes erschienen hohe a-Wellen (Abb. 5). Nach Noradrenalin-Verabfolgung kam es abermals zur Rhythmusänderung der proximalen Kammer und die Kontraktionen erfolgten — ebenso wie die einer einheitlichen rechten Kammer — synchron mit dem distalen Kammerabschnitt, im Zeitpunkt der Kammersystole. Der systemische Blutdruck normalisierte sich zu diesem Zeitpunkt, nach Abklingen der Noradrenalinwirkung wiederholte sich jedoch die vorangehend beschriebene Erscheinung. Durch wiederholte Noradrenalingabe konnte die erwähnte partielle Dissoziation der rechten Kammer behoben werden, zur Aufrechterhaltung dieses Zustandes war jedoch Noradrenalininfusion erforderlich. Angesichts des beschriebenen Zustandes traten wir von der Fortsetzung der Operation zurück und schlossen den Brustkorb.

Der erforderliche Blutdruck (80 mmHg) war auch weiterhin nur mittels Noradrenalininfusion zu sichern; der spontane Blutdruck des Patienten normalisierte sich lediglich in der 68. postoperativen Stunde. 30 Tage später wurde der Kranke ohne postoperative Komplikationen mit kompensiertem Kreislauf entlassen.

Besprechung

Die bei EBSTEINscher Anomalie auftretenden verschiedenen Rhythmusstörungen wurden von mehreren Verfassern beobachtet [2, 3, 5, 6, 7], und es wird angenommen, daß für den tödlichen Ausgang ebenfalls die Rhythmusstörungen verantwortlich sind. WOOD [6], sowie LEV und Mitarbeiter [3] beobachteten bei EBSTEINscher Anomalie mehrmals das Auftreten des W—P—W-Syndroms. In einem Fall von LEV war es histologisch nachzuweisen, daß der rechte atrioventrikuläre Ring den rechten Vorhof von der Kammer nicht vollkommen absonderte. Zwischen den beiden Herzhöhlen fand sich ein intermediäres, spezifisches Gewebe enthaltendes Bündel. Diese Fasern sicherten zwischen dem Sinusknoten und dem rechten TAWARAschen Schenkel durch erhaltene Kontinuität eine direkte Verbindung. KJELLBERG und Mitarb. [2] teilten ihre bei EBSTEINschen Anomalie-Fällen ermittelten elektromyographischen Befunde der rechten Vorhofkontraktionen in 3 Gruppen ein: In 2 von 6 Fällen war der Maximalausschlag des rechten Vorhofs nach dem QRS-Komplex sichtbar. In der auf Trikuspidalinsuffizienz charakteristischen Wellenbildung nahm das rechte Aurikel nicht Teil. In weiteren 2 Fällen erstreckte sich der Maximalausschlag des rechten Vorhofs vom Beginn der Q-Welle bis zum Ende der R-Welle. In 1 Fall war das Kymogramm des rechten Vorhofs und des atrialisierten rechten Kammerabschnitts gänzlich verschieden. Während der Kammersystole zeigte der rechte Vorhof Initialfüllung, während der Diastole dagegen rasche Füllung. Die Kurve entsprach einer raschen Kammerdilatation und einer Maximalfüllung der rechten proximalen Kammer.

Werden unsere vom rechten und linken Vorhof aufgenommenen Druckkurven verglichen, so ergibt sich folgendes: Während die a-Wellen des rechten Vorhofs (Abb. 2) kaum zu erkennen sind, treten die a-Wellen des linken Vorhofs deutlich in Erscheinung (Abb. 4). Auf der Kurve des linken Vorhofs sind als Zeichen des Mitralklappenschlusses außer den c-Wellen auch große av-Wellen sichtbar. Auf der Kurve des rechten Vorhofs waren analoge Wellen nicht zu beobachten.

Die Rhythmusveränderungen, die es zur Folge hatten, daß die Kontraktionen der proximalen rechten Kammer mit den Vorhofkontraktionen simultan

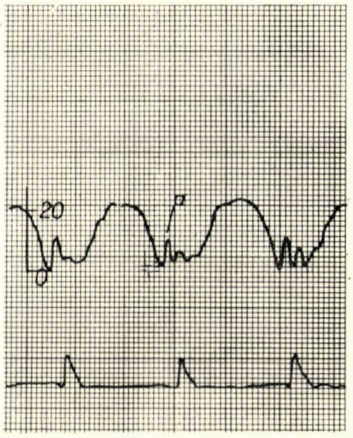

Abb. 6. Intraoperative Druckkurve des distalen rechten Kammerabschnitts während der simultanen Depolarisation des rechten Vorhof-proximalen Kammerabschnitt-Komplexes

verliefen, änderten die Hämodynamik des rechten Herzens grundlegend. Die Druckverhältnisse der aus dem betreffenden Kammerabschnitt und dem rechten Vorhof bestehenden Höhle entsprachen einer typischen Trikuspidalinsuffizienz. Auf die hohen a-Wellen folgten der Kammerejektion entsprechende systolische Druckerhöhungen (Abb. 5), und in der erwähnten Herzhöhle stieg der Druck an. Als Resultat dieses Zustandes trat — wie bereits erwähnt — eine systemische Druckabnahme auf. Der Steigerung der Trikuspidalinsuffizienz entsprechend erhöhte sich der Druckwert des eine funktionelle Einheit darstellenden rechten Vorhof-proximalen rechten Kammer-Komplexes während der Systole des distalen rechten Kammerabschnitts von dem vorangehenden 17 mmHg auf 22 mmHg. Auf der Druckkurve der distalen Kammer waren zur Zeit der Dissoziation während der Diastole ungewöhnliche a-Wellen zu beobachten, die dem vergrößerten systolischen Volumen des rechten Vorhofs zuzuschreiben sind (Abb. 6).

Folgerung

Im Mangel an histologischen Befunden können wir die beobachteten klinischen Erscheinungen vom anatomischen Gesichtspunkt schwer erklären. Anhand der in der Literatur mitgeteilten indirekten Angaben [2, 3] nehmen auch wir die Möglichkeit der zwischen dem Sinus und dem rechten TAWARA-schen Schenkel bestehenden direkten Verbindung an. Diese Supposition scheint auch die Beobachtung von DATEY und GANDHI [5] zu unterstützen, die bei EBSTEINscher Anomalie in der atrialisierten proximalen rechten Kammer mittels Elektrodenkatheter Vorhoffibrillation registrierten. In 1 Fall von KJELLBERG wies das Elektrokymogramm auf die gemeinsame Füllung des rechten Vorhofs und der proximalen rechten Kammer; diese Erscheinung entsprach wahrscheinlich ebenfalls einer Rhythmusänderung.

Schließlich könnte angenommen werden, daß für die plötzlichen Todesfälle ebenfalls Rhythmusveränderungen verantwortlich sind.

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THE EFFECT OF AMMONIUM CHLORIDE ON THE RNA CONTENT OF THE RAT LIVER

By

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(Received March 11, 1966)

Ammonium chloride in a dose of 0.1 g was administered to rats every second day over a period of twenty days. Subsequent histochemical staining revealed increased pyroninophilia, electron microscopic study a richness in ergastoplasm in the liver cells. A 24.8 per cent increase of hepatic RNA contents as compared to the controls was demonstrated by a quantitative method. The findings suggest ammonium chloride treatment to lead to increased protein synthesis in the liver.

The mechanism of action of ammonium chloride, particularly in respect to its acidifying character, has been a subject of study from the beginning of the century. As early as 1900 Pohl and Münzer [16] have shown that ammonium chloride caused acidosis. Haldane [9] induced acidosis in a self-experiment by ingestion of a massive dose of ammonium chloride [9], believing acidosis of this origin to be due to splitting of ammonium chloride into NH_3 and HCl, the first being assumed to be converted in the liver to urea and the second — i.e. the remaining HCl radical — to be the factor responsible for acidosis. Though Haldane's hypothesis was later modified by Winterstein and Göckhahn, Jacobs, Loeschke and Sugioka [22, 10, 13], NH_3 is still assumed to be broken down or transformed mainly in the liver. Fazekas [4, 5, 7] investigated the effect of ammonium chloride, ammonium sulphate, ammonium carbonate and other salts of this type in rabbits. The animals treated with these substances displayed enlargement of the anterior pituitary (with an increased basophile cell population), as well as of the ovaries and the uterus, furthermore follicular maturation, production of corpora lutea, cyclic changes of the uterine horns, broadening and hyperfunction of the adrenal cortex, and a considerable increase in body weight. The ammonium salts were thus assumed to start a chain of enhanced anabolic processes.

The present histochemical, electron microscopic, and biochemical studies deal with the effect of ammonium chloride on the liver.

Material and methods

Inbred male albino rats of 250 g body weight kept on a standard diet were given 0.1 g ammonium chloride as a 5 per cent solution through a gastric tube every second day on ten occasions, i.e. over twenty days. On the 20th day samples of the liver were excised under hexo-

barbital anaesthesia from 10 treated and 10 untreated animals. The specimens were fixed in CARNOY, washed with alcohol, embedded in paraffin and 10 μ thick sections were prepared and stained with methylgreen-pyronine. Specimens measuring 1 cu. mm from the same animals were fixed in 1 per cent osmium [14] for one hour at 4 °C, and after gradual dehydration with alcohol were embedded in Araldit; sections made with an LKB type ultramicrotome were contrasted according to REYNOLDS [17]. The sections were examined and photographed by means of a Tesla 242 D type electron microscope (Electron Microscopic Laboratory, József Attila University, Szeged).

In 350 to 450 mg specimens from the liver of each animal the RNA content was estimated by the method of SCHNEIDER [20], SCHMIDT and THANNHAUSER [19], as modified by ÖRDÖG and DOBOZI [15].

Results

In agreement with THEMANN and STICHNOTH [21], in healthy liver tissue around 25 per cent of the cells showed slightly increased pyroninophilia (Fig. 1).

After treatment with ammonium chloride increased pyroninophilia was

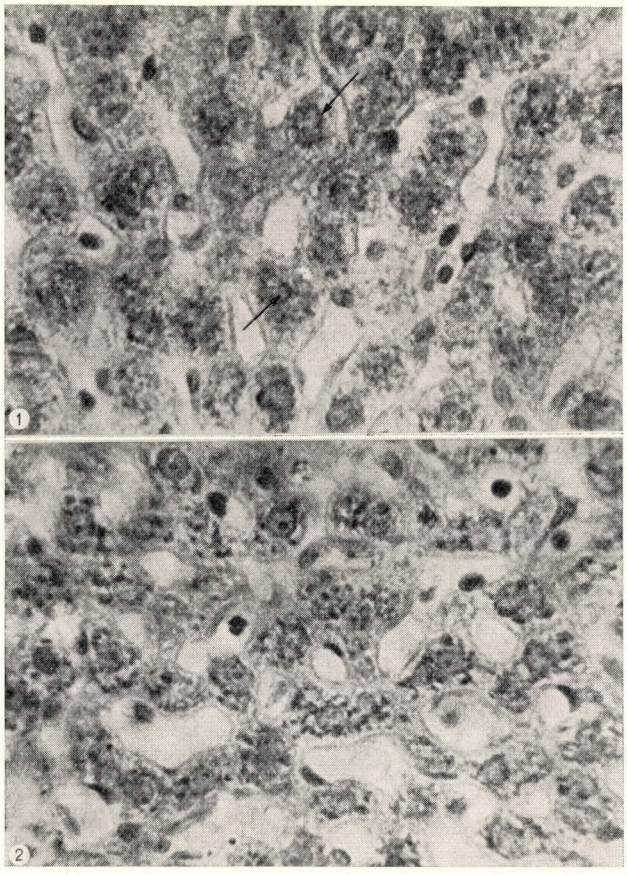
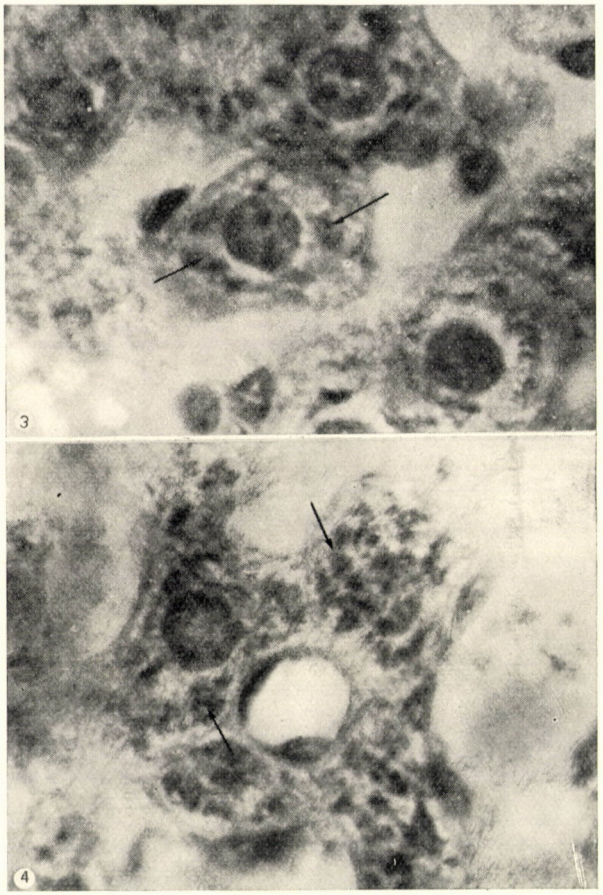


Fig. 1. Rat liver. Methylgreen-pyronine staining. The arrows indicate liver cells with increased pyroninophilia. × 650

Fig. 2. Rat liver after treatment with ammonium chloride. Methylgreen-pyronine staining. The liver cells filling the visual field show increased pyroninophilia. × 650

found in all the cells (Fig. 2). Coarse pyronine granules were clearly seen in the liver cells under high power (Figs 3, 4).

These results led us to the electron microscopic study of the possible ultrastructural changes. No difference in this respect was, however, demonstrable



Figs 3, 4. Rat liver after treatment with ammonium chloride. Methylgreen-pyronine staining. The arrows indicate coarse pyronine granules. × 1500

between treated and untreated animals. The typical cytoplasmic elements, such as the mitochondria, lysosomes, Golgi's apparatus, had a normal appearance. On the other hand, marked changes were observed in the ergastoplasm. While in the controls there was, in accordance with the findings in the methylgreen-pyronine-stained preparations, a fully developed ergastoplasm in 25 per cent of the cells, in the material derived from animals after treatment with ammonium chloride, a rich ergastoplasm had become manifest in practically

all of the cells. It was remarkable in the treated animals that the coarse endoplasmic reticulum assumed a characteristic lamellar pattern in nearly all the cells, a finding pointing to increased protein synthesis [8]. Further ultrastructural signs of an enhanced protein synthesis, such as the ribosome-helices described by RÖHLICH et al. [18], were absent.

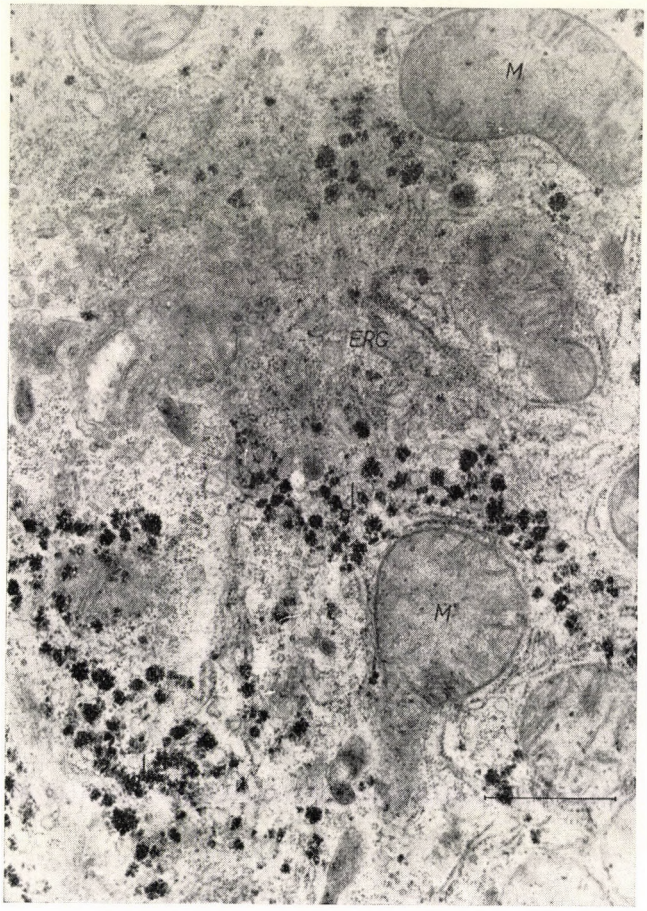


Fig. 5. Electron microscopic detail of a rat liver cell. M: mitochondria; gl: glycogen; ERG ergastoplasm. × 27.000

Thus, ammonium chloride led to profound changes in the RNA content of liver cells. In order to assess the degree of increase of RNA content, it was estimated by a biochemical quantitative method both in the treated animals and in the controls, in specimens of the liver of the same animals which had served for the histochemical and electron microscopic investigations. (According to preliminary experiments, no correct evaluation would have been otherwise

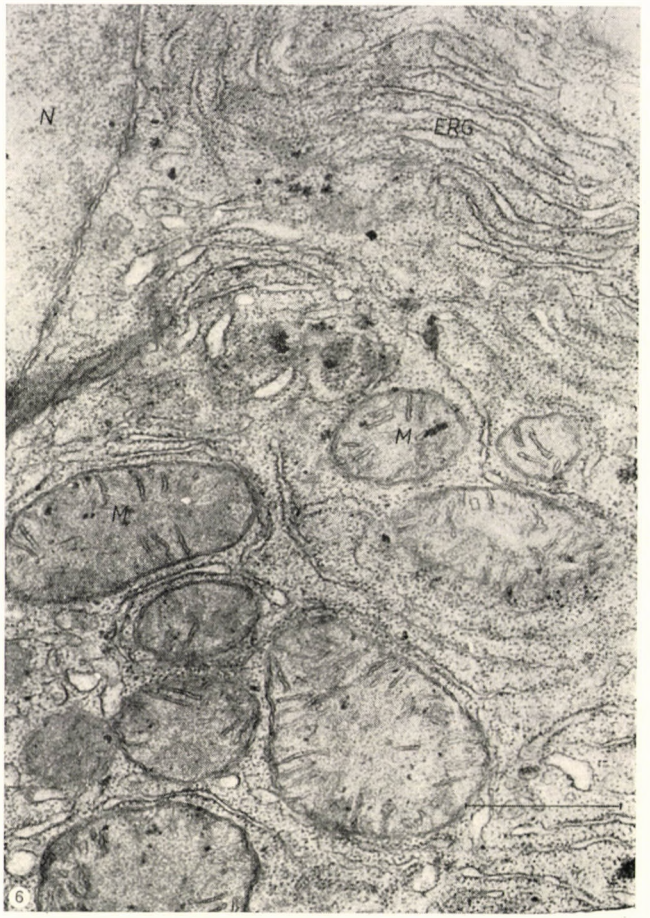


Fig. 6. Electron microscopic detail of rat liver cell after ammonium chloride treatment. Lamellar pattern of ergastoplasm (ERG). M: mitochondria; N: nucleolus. × 32.000

Table I

RNA-content of liver of rats treated with ammonium chloride

No	1	2	3	4	5	6	7	8	9	10
RNA γ/g	7471	8179	7419	8041	7651	7951	8597	8172	8984	8734
Avèrage	8120±529									

Table II

RNA-content of liver of control rats kept on standard diet

No	1	2	3	4	5	6	7	8	9	10
RNA γ/g	6472	6612	6176	6204	6860	5916	6640	6680	6648	6816
Average	6502.4 ± 312									

possible.) Results are shown in Tables I and II. The RNA values refer to moist weight. It clearly emerges from the Figures that the RNA content in the liver of treated animals was 1618 μg more than in the control livers. This corresponded to a 24.8 per cent deviation; the difference was significant statistically ($p < 0.01$; $t_{18} = 8.374$).

Discussion

On the evidence of studies quoted in the introductory section of this paper, ammonium chloride has been found to cause a great variety of systemic changes. Our attention has been directed to ammonium chloride by FAZEKAS [6] who claimed the substance to be a general roborant.

As demonstrated by LOESCHKE and SUGIOKA, ammonium chloride introduced into the organism yields ammonia which penetrates the cellular membrane and displays its action intracellularly. Massive accumulation of this substance carries a high toxicity, therefore the organism mobilizes several mechanisms for its elimination. It is generally accepted (HALDANE and others) that the greatest part of ammonia is converted into urea through a specific process which takes place in the liver as part of the ornithine-cycle [12].

The reason for our using animals of identical breed, age, body weight, and sex, and for keeping them on a standard diet, was a well-known fact that the RNA content of the tissues including the liver shows a wide variety depending on species, sex, age, and dietary conditions. Neglect of these factors may be a source of errors.

On the ground of morphological and biochemical evidence it has been shown that treatment with ammonium chloride leads to an increase in the RNA content of liver cells.

According to BRACHET [1] and DAVIDSON [3], enhanced protein synthesis is associated with an accumulation of RNA. The increased pyroninophilia and the richness in ergastoplasm may be regarded as morphological evidence of an increased RNA content in the liver. Quantitative measurements showed this increase to have amounted to 24.8 per cent, representing a statistically significant ($t < 0.01$) difference as compared to the controls. According to CANELLAKIS [2], the RNA molecules take part in protein synthesis as amino acid carriers, transferring the amino acids to the microsomes in order to be built into the microsomal proteins.

The increased RNA values found in the present study are interpreted as a sign of an enhanced intrahepatic protein synthesis; this might account for the anabolic effect of ammonium chloride.

Further studies are in course to ascertain whether ammonium chloride treatment leads to an increase of the RNA content in other tissues as well.

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HAEMODYNAMIC EFFECTS OF EXTEROCEPTIVE STIMULI IN RATS IN THE WAKING STATE AND WITH GROLLMAN-HYPERTENSION

By

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The haemodynamic changes in waking and anaesthetized rats with GROLLMAN-hypertension and in normal controls, respectively, may be resumed as follows.

1. The circulation of waking rats as compared to that of anaesthetized controls is characterized by a considerable increase in cardiac output and a shifting of its organ fractions, in favour of the carcass at the expense of the renal and splanchnic fractions.

2. Proper evaluation of the haemodynamic changes consequent upon the production of GROLLMAN-hypertension was rendered difficult by the stress of excitation when the animals were awake and by a substantial fall of blood pressure in the hypertensive animals while under general anaesthesia. The results suggest, however, that the increase of cardiac output may play a part in the production of GROLLMAN-hypertension while its shifting may be inferred to from the increased coronary fraction found in the waking hypertensive group and from the high fraction for the carcass in the anaesthetized hypertensive groups.

There is ample evidence in support of the theory which links up the production of hypertension with an increased peripheral resistance. A rise in cardiac output has also been claimed to play a part in the increase of blood pressure in human beings as well as in laboratory animals, particularly in the earlier stages of hypertensive disease (FISHBERG [1954], BROD et al. [1959], BROD [1960], SZABÓ [1963], LEDINGHAM and COHEN [1963], FINKIELMAN et al. [1965]). The renal haemodynamic changes have been studied extensively and it has been accepted that essential hypertension leads to a reduction of renal blood flow and to a parallel increase in renal vascular resistance. According to BROD (1960), reduced renal blood flow is associated with an increased blood supply of the musculature. Information is scarce concerning haemodynamic conditions prevailing in the other organs.

In order to gain insight into the mechanism of haemodynamic changes associated with hypertensive disease, it is essential to study the haemodynamic conditions of the different organs apart from cardiac output and total peripheral resistance of which they are, actually, the determining factors. We have approached the problem by the use of SAPIRSTEIN's isotope indicator dilution technique in various types of induced hypertonia. We had, however, to

cope with methodological difficulties, such as a fall of blood pressure to nearly normal levels in rats with GROLLMAN-hypertension while under pentobarbital anaesthesia. Another difficulty was that even in the alert controls cardiac output and its distribution showed deviations beyond the normal range. In the present studies the haemodynamics of rats with GROLLMAN-hypertension were compared with those of alert restrained and non-restricted controls and of rats under pentobarbital anaesthesia.

Methods

Hundred inbred male rats weighing between 131 and 255 g were used. Average body weight within the groups ranged from 141 g to 184 g. After 16 to 20 hours fasting intraperitoneal anaesthesia was carried out with 50 mg/kg of pentobarbital, 15 to 30 minutes prior to the haemodynamic studies.

Hypertension was induced by GROLLMAN's method (1944), i.e. after unilateral nephrectomy, ischaemia of the other kidney was induced by a figure-of-eight-ligature. Development of hypertension was followed by indirect blood pressure readings using the device described by GÁTI et al. (1959).

Cardiac output was determined by the dye dilution technique, using Evans blue, and the organ fractions of cardiac output by the SAPIRSTEIN's isotope indicator fractionation technique (1956, 1958), using ^{86}Rb. Blood flow and peripheral resistance of the organs were computed from the values of blood pressure, cardiac output and its organ fractions. Details of these procedures have been described previously (KÁLLAY and TAKÁCS [1961], TAKÁCS et al. [1962]).

The layout of the experiments was as follows:

a) Correlation of haemodynamic changes in anaesthetized and alert controls and in alert animals with GROLLMAN-hypertension four weeks after the operation. These animals were not restricted during the haemodynamic tests, cardiac output was not determined, ^{86}Rb was given into the tail vein.

b) Three groups of animals similar to a), restrained in the supine position. In these groups, cardiac output was determined, blood pressure was measured directly, the solutions were given into the femoral vein after previous infiltration of the incision sites with 0.5 ml of 1 per cent procaine solution, as the total dose.

Table I

Deviation from control

No symbol	not significant	$P > 0.05$
·, *	significant	$P < 0.05$
··, **	markedly significant	$P < 0.01$
···, ***	highly significant	$P < 0.001$

Units

Cardiac output	ml/min/100 g body weight
Blood pressure	mm Hg
Resistance/body	10^3 dyn. sec. cm^{-5}/100 g body weight
Blood flow	ml/min/100 g organ weight
Resistance/organ	10^3 dyn. sec. cm^{-5}/100 g organ weight
Fraction	Blood flow of total organ in percentage of cardiac output

c) Groups with one, two, and four weeks' hypertension correlated with sham-operated controls. All determinations were carried out after pentobarbital anaesthesia and immobilization.

Further details will be dealt with together with the results.

Results were evaluated with STUDENT's two-sample "t"-test. In the tables the common scatter has been tabulated, since this did not affect evaluation of the results.

The symbols and units of measurement used in the tables and graphs are shown in Table I.

Results

a) *Unrestricted anaesthetized and alert controls and waking animals with* GROLLMAN-*hypertension*

The animals were wrapped up in a cloth and fixed, care being taken to maintain them in their natural posture. The necessary solutions were injected

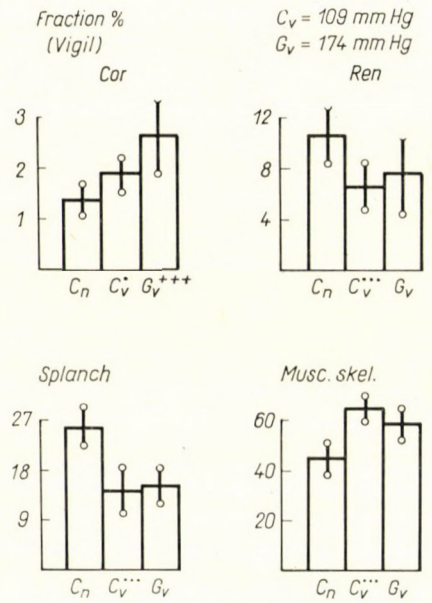

Fig. 1. Coronary (cor), renal (ren), splanchnic (splanch), and carcass (musc. skel.) fractions of cardiac output in control rats under pentobarbital anaesthesia (C_n), in waking, unrestricted control rats (C_v) and in waking rats with GROLLMAN-hypertension (G_v)

n to the tail vein. Mean values obtained by indirect blood-pressure readings were 110 mm Hg prior to the GROLLMAN's operation, and 172 mm Hg four weeks later prior to the haemodynamic tests. Table II and Fig. 1 show the organ fractions of cardiac output. — In the waking group, correlated with the anaesthetized controls, the fractions for the coronary system and the carcass were higher, those for the kidney, liver and intestines lower, than in the controls. In the hypertensive group, as compared to the alert controls, the coronary frac-

Table II

Organ fractions of waking unrestricted rats after Grollman's operation

Number of cases (n)	Controls (pento-barbital)	Waking groups		Within-sample standard deviation
		Controls	Grollman-operation	
	10	10	10	
		Mean values		
Heart	1.4	1.8·	2.7***	0.4
Kidney	10.9	6.7···	7.8	2.5
Lung	3.0	3.4	4.2	1.1
Liver	8.4	6.3··	7.5	1.5
Intestines	17.7	8.3···	8.2	2.7
Splanchnic area (liver + intestines)	26.1	14.6···	15.7	3.5
Skin	13.4	13.0	14.3	4.4
Carcass	45.0	60.6···	59.4	5.1

Symbols

· = significant as compared with the controls anaesthetized with pentobarbital
* = significant as compared with the waking controls.

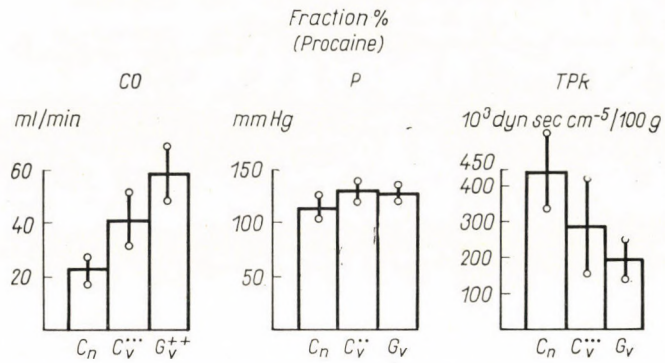

Fig. 2. Cardiac output (CO), direct blood pressure readings (P) and total peripheral resistance (TPR) in control rats under pentobarbital anaesthesia (C_n), in waking, immobilized controls (C_v) and in waking, restricted rats with GROLLMAN-hypertension (G_v)

tion further increased while the other organ fractions remained practically unchanged.

b) *Immobilized animals under local anaesthesia; anaesthetized control group; waking control group; group with* GROLLMAN-*hypertension* (Table III, Figs 2 and 3)

The incision sites were infiltrated with procaine.

Mean blood-pressure in the waking controls and in the groups with GROLLMAN-hypertension prior to the tests were 110 and 186 mm Hg respectively. Direct blood-pressure readings in supine immobilized animals yielded mean values of 132 and 131 mm Hg respectively, significantly higher than the 117 mm Hg mean for the anaesthetized control group.

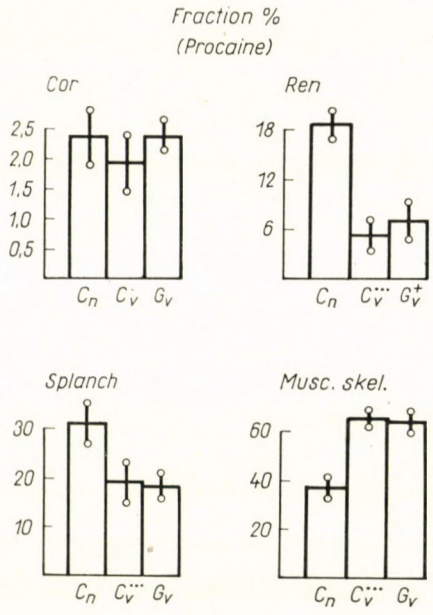

Fig. 3. Coronary (cor), renal (ren), splanchnic (splanch) and carcass (musc. skel.) fractions of the cardiac output in control rats under pentobarbital anaesthesia (C_n), in waking, restricted controls (C_v) and in waking restricted rats with GROLLMAN-hypertension (G_v)

Cardiac output in the waking control group considerably exceeded that in the anaesthetized control group, while total peripheral vascular resistance in the former group was distinctly lower than in the latter. Changes in organ blood flow were inconsistent in the alert group, renal blood flow was considerably reduced, hepatic and splanchnic flow was unchanged, pulmonary and dermal flow rose to double, that of the carcass to triple the normal value. Peripheral resistance of the coronary, hepatic, and splanchnic areas remained unchanged, in the renal area it rose excessively, while in the skin, lungs and carcass it diminished.

The figures were clearly indicative of a redistribution of cardiac output, as reflected by the slight reduction in the coronary fraction, a considerable one in the renal and splanchnic fractions, and a rise in the fraction for the carcass.

Cardiac output in the group with GROLLMAN-hypertension was even higher than in the waking control group, while the peripheral resistance showed

Table III

Circulation of waking, restricted rats after Grollman's operation

Number of cases (n)	Controls (pento-barbital)	Waking groups		Within-sample standard deviation
		Controls	Grollman-operation	
	10	15	8	
		Mean values		
Total body				
Cardiac output	22.0	42.0···	57.1**	11.1
Blood pressure	117.0	131.7··	130.6	11.3
Resistance	445.4	276.8···	189.8	108.5
Heart				
Blood flow	158.9	220.8	401.6***	79.1
Resistance	63.5	62.9	26.7**	29.9
Fraction	2.3	1.9·	2.3	0.5
Kidney				
Blood flow	382.5	237.7··	310.3	102.3
Resistance	26.8	52.3··	35.2*	17.2
Fraction	18.4	5.4···	7.4*	1.8
Lung				
Blood flow	57.4	105.0··	110.8	36.3
Resistance	195.4	113.4··	102.7	64.0
Fraction	3.2	3.0	3.0	0.8
Liver				
Blood flow	53.2	56.0	79.2**	19.2
Resistance	191.1	211.3	139.7*	64.2
Fraction	10.5	7.0···	6.7	1.4
Intestines				
Blood flow	66.2	68.0	87.7	24.5
Resistance	149.7	177.0	125.4*	51.3
Fraction	20.5	11.3···	11.2	3.1
Splanchnic area (liver + intestines)				
Fraction	31.0	18.3···	17.9	3.7
Skin				
Blood flow	8.6	15.8··	26.2***	5.5
Resistance	1149.6	792.3·	425.8*	365.6
Fraction	7.7	7.8	8.7	1.1

Number of cases (n)	Controls (pento-barbital)	Waking groups		Within-sample standard deviation
		Controls	Grollman-operation	
	10	16	8	
Carcass		Mean values		
Blood flow	12.3	42.4···	55.7*	12.0
Resistance	81.0	29.2···	19.6	16.0
Fraction	37.0	64.3···	63.7	4.7

Symbols

· = significant as compared with anaesthetized controls.
* = significant as compared with waking controls.

no significant difference between the two groups. In the hypertensive group, blood flow in all of the organs was higher than in the waking controls, though this difference did not always reach statistical significance. Peripheral resistance in the organs was found to diverge in the opposite direction; the differences were significant with the exception of the fraction for the carcass. Apart from a slight rise in the renal fraction, the other organ fractions remained unchanged.

c) *Restricted anaesthetized rats; sham-operated controls; hypertensive groups one, two, and four weeks after* GROLLMAN*'s operation*

Since the circulatory parameters one to four weeks after operation showed no difference, in Table IV the data for rats with two weeks' hypertension have only been assembled.

Indirect blood pressure readings in waking animals two weeks after the GROLLMAN'S operation showed a significant elevation (186 mm Hg) as contrasted with the sham-operated controls (110 mm Hg). Under anaesthesia, however, the differences were not significant, the mean value for the hypertensive group being only 12 mm Hg higher than in the control group.

It emerges from Table IV that under anaesthesia, the two groups showed practically no differences. The slightly reduced hepatic fraction and the slightly increased blood flow and the fraction for the carcass in the hypertensive group failed to attain statistical significance.

Discussion

Circulation in the waking rats showed considerable differences as compared to anaesthetized animals. In the first waking group where the animals had been fixed in their natural posture, the distribution of cardiac output di-

Table IV

Circulation of anaesthetized rats after Grollman's operation

Number of cases (n)	Controls (sham-operation)	Two weeks after Grollman-operation	Within-sample standard deviation
	7	10	
Total body		Mean values	
Cardiac output	31.0	34.3	6.8
Blood pressure	105.7	117.5	13.8
Resistance	277.3	281.1	84.9
Heart			
Blood flow	181.0	195.4	49.7
Resistance	49.9	49.8	17.8
Fraction	1.9	2.0	0.4
Kidney			
Blood flow	422.6	400.2	111.7
Resistance	20.9	24.4	9.2
Fraction	17.3	18.8	3.5
Lung			
Blood flow	67.1	80.5	26.0
Resistance	141.2	126.9	73.1
Fraction	3.4	2.9	0.8
Liver			
Blood flow	64.8	65.3	19.9
Resistance	132.8	153.2	58.3
Fraction	9.9	8.4·	1.5
Intestines			
Blood flow	98.7	117.2	26.9
Resistance	86.9	83.2	33.3
Fraction	21.3	24.7	3.6
Splanchnic area (liver + intestines)			
Fraction	31.2	33.1	3.7
Skin			
Blood flow	11.6	11.7	2.9
Resistance	768.1	852.4	254.2
Fraction	6.9	6.4	1.1
Carcass			
Blood flow	18.3	23.7·	4.1
Resistance	47.6	40.7	14.7
Fraction	39.2	46.1··	4.8

verged significantly from that of the anaesthetized control group; the coronary and carcass fractions were increased, the renal and splanchnic fractions were reduced. Practically the same shifting was registered in waking animals which had received the injections through a cannula previously inserted into the jugular vein (KÁLLAY and TAKÁCS [1961]).

This series had the shortcoming that only part of the intended determinations were possible within its limits. In the second alert group where the necessary surgical interventions had been carried out under local anaesthesia, cardiac output was also determined and blood pressure was measured directly. Despite local anaesthesia, the stress was presumably greater in this group than in the previous one.

Though reduction of peripheral resistance was considerable, blood pressure, correlated with that of the controls, showed an increase in consequence of the increase in cardiac output. It must be pointed out that renal blood flow diminished in spite of the markedly elevated cardiac output, together with a considerable increase of flow in the carcass. This suggests that haemodynamic changes occurring in waking animals under the stress of excitation, are characterized by hypertension and a shifting of cardiac output, resulting in an increased fraction for the carcass, largely at the expense of the kidneys and, to a lesser degree, of the splanchnic area (Figs 1 and 3). These changes corresponded to those observed by BROD et al. (1955) in humans under mental stress or those recorded in laboratory animals after bleeding. The excessive increase of flow in the carcass, though sufficiently accounted for by an increased muscle tone, need not be associated with it, as demonstrated after blood loss in rats (TAKÁCS et al. [1962]). The considerable reduction of the renal fraction shows that under excitation shifting takes place at the expense of renal flow.

The experiments in animals with GROLLMAN-hypertension confronted us with considerable methodological difficulties. In the waking animals it was the stress of excitation which caused profound haemodynamic changes, while in the anaesthetized group, hypertension practically ceased under the effect of pentobarbital. These difficulties restrict us to very guarded conclusions though five hypertensive groups have been studied. The increase of cardiac output in the waking hypertensive procainized group might be interpreted as being contributive to the production of GROLLMAN-hypertension. Similar results have been recorded by other investigators in experimental and clinical hypertension (FISHBERG [1954], BROD et al. [1959], BROD [1960], FINKIELMAN et al. [1965], LEDINGHAM and COHEN [1963]). Shifting of cardiac output in hypertension is suggested by two data emerging from the present experiments, i.e. the increased coronary fraction in the waking unrestricted animals and the higher fraction for the carcass in the anaesthetized group.

Though the present investigations have failed to clarify the problem under study, i.e. that of a shifting of cardiac output in hypertension, they have

been, nevertheless, informative in certain respects, by yielding comparative data as regards the haemodynamic conditions in waking and in anaesthetized animals.

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STUDIES IN EXPERIMENTAL PULMONARY OEDEMA

I. PATHOMECHANISM OF PULMONARY OEDEMA INDUCED BY HYPEROXYGENATION

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It has been shown that on increasing pO_2 in high pressure O_2 atmosphere, survival of rats is reduced and the induction of pulmonary oedema accelerated. Hypercapnia develops successively in the muscles, the brain, and the liver, pulmonary oedema being subsequent to these changes in the majority of the animals. The presence of CO_2 promotes the ill-effects of high pressure O_2 by accelerating the induction of pulmonary oedema.

The influence of hyperoxygenation on the organism has long been a subject of research. It has been shown that protracted inhalation of a gas mixture of atmospheric or higher pressure with a 60 per cent or higher O_2 content leads to pulmonary oedema [1, 2, 3]. The mechanism underlying this process is, however, unknown though various angles of approach have been sought. Apart from local bronchopulmonary damage incriminated by PENROD [4], neuroendocrine, sympathoadrenal [5, 6] as well as central nervous [7] influences have been studied as possible pathogenetic factors. Tissue hypercapnia secondary to hyperoxia has been held by certain authors [8, 9] to be instrumental in bringing about pulmonary oedema of this type. POULSEN [10] and PRAUSNITZ [11] even claim that CO_2 alone may account for its induction. In our earlier experiments [12] we have, however, failed to induce pulmonary oedema in dogs by inhalation of air of a high CO_2 concentration under atmospheric pressure.

The present studies were intended to throw light on the part played by tissue hypercapnia in the pathomechanism of pulmonary lesion due to hyperoxygenation.

Method

90 inbred rats weighing between 180 and 300 g were used.

To study the effect of pure O_2 at high pressure the animals were placed in pressure chambers at 3 and 4 atm. respectively. The exhaled CO_2 was absorbed on soda-lime, in this manner the CO_2 concentration in the chambers never exceeded 2 or 2.5 vol per thousand. The animals could be observed through the window of the chamber during the whole course of the experiment. In one group the time of spontaneous death was recorded while the other groups were kept in high pressure O_2 for 2, 3, 4, or 5 hours and then killed by decapitation. The extent of

pulmonary damage in these animals as well as in those which had died spontaneously was inferred from histologic evidence and from the index, 100 × lung weight per total body weight. To assess the degree of tissue hypercapnia the CO_2 content of brain, liver and muscle tissues was determined in each animal by the modified ANREP-technique [13]. The CO_2 content of the chamber was estimated by the Department of Air Hygiene of the Institute of Public Health.

In a further phase of the studies, the animals were kept in high pressure O_2 at 3 atm. with 4 per cent CO_2 for two hours, then decapitated. The CO_2 content of the tissues and the extent of pulmonary damage were assessed in the manner described above.

Results

In normal animals the lung weight per total body weight index averaged 0.85; the CO_2 content of brain tissue, 15.1 mM/kg; of liver tissue, 19.8 mM/kg; of muscle tissue 16.1 mM/kg. The results obtained in the control animals and

Table I

Tissue CO_2 concentration and lung weight per total body weight index in rats after exposure to 3 atm. O_2 atmosphere

Time of exposure	mM/kg CO_2 in			Lung weight/total body weight
	Brain	Liver	Muscle	
Normal value	15.1±1.06	19.8±0.92	16.1±0.99	0.85±0.04
2 hrs.	15.9±1.01 p=0.7	18.4±1.56 p=0.75	16.3±1.57 p=0.35	0.99±0.06 p>0.05
3 hrs.	18.3±1.22 p<0.1	18.3±1.47 p=0.78	27.7±5.61 p=0.06	1.12±0.06 p<0.01
4 hrs.	22.2±1.17 p<0.01	26.2±2.41 p=0.03	29.7±1.94 p<0.001	0.92±0.16 p>0.2
5 hrs.	22.9±0.67 p<0.01	22.5±1.87 p=0.04	28.4±2.08 p=0.001	1.20±0.13 p=0.001
6 hrs. 18 min.	35.7±2.59 p≪0.001	36.3±1.03 p<0.001	39.9±2.25 p≪0.001	1.27±0.14 p≪0.001

in those exposed to 3 atmosphere O_2 for various lengths of time are shown in Table I. Average survival time under such pressure was 6 hrs. 18 min. In the animals which had died spontaneously distinct hypercapnia was demonstrable in all of the examined tissues. The lungs showed microscopic evidence of diffuse

alveolar filling with oedema fluid, and the lung weight per body weight index was 1.27.

After 2 hours of exposure, tissue CO_2 content was found unchanged and the index slightly elevated (0.99) within the limits of significance. In 7 out of 20 animals signs of incipient pulmonary oedema were found.

At 3 hours, muscular hypercapnia (27.7 mM/kg) was distinct while in the brain the CO_2 content was only slightly elevated (18.3 mM/kg) and in the liver

Table II

Tissue CO_2 concentration and lung weight per total body weight index in rats after exposure to a 4 atm. O_2 atmosphere

Time of exposure	mM/kg CO_2 in			Lung weight/total body weight
	Brain	Liver	Muscle	
Normal value	15.1±1.06	19.8±0.92	16.1±0.99	0.85±0.04
2 hrs.	19.6±1.44 p=0.2	22.3±2.75 p=0.05	24.1±4.06 p=0.2	0.94±0.09 p=0.06
3 hrs. 47 min.	37.8±2.40 p≪0.001	40.5±2.87 p≪0.001	44.2±4.69 p<0.001	1.45±0.16 p≪0.001

it was normal. The histologic picture of the lungs was similar to that found in the previous group.

At 4 hours, the CO_2 content was significantly increased in brain, liver and muscle tissue (22 mM/kg, 26.2 mM/kg, and 29.7 mM/kg, respectively), while the lungs showed no additional change.

At 5 hours, the lung index as well as its histologic inspection revealed gross pulmonary oedema in all of the animals while the tissue CO_2 level showed no additional increase. Excessive tissue hypercapnia was demonstrable in the terminal stage (brain, 35.7 mM/kg; liver, 36.3 mM/kg; muscle, 39.9 mM/kg).

In 4 atm. O_2, average survival time was reduced to 3 hrs. 47 min., and though the exposure was relatively short, yet it was sufficient to allow pulmonary damage (average index 1.45) and excessive hypercapnia (brain, 37.8 mM/kg; liver, 40.5 mM/kg; muscle, 44.2 mM/kg) to develop (Table II).

After 2 hours of exposure the index averaged 0.94, and histologic evidence of pulmonary oedema was found in 8 out of 20 animals. CO_2 concentration in the tissues was slightly elevated (brain, 19.6 mM/kg; liver, 22.3 mM/kg; muscle, 24.1 mM/kg); it was highest in the muscle again.

Analysis of the additional effect of CO_2 in hyperoxygenation revealed that the presence of 4 per cent CO_2 in O_2 at a pressure of 3 atm. resulted in gross

or moderate pulmonary oedema with an index of 1.26 in the majority of the animals even after two hours exposure. Survival was considerably reduced, 3 out of 10 animals having died within two hours. CO_2 concentration in the tissues was excessive in all of the animals (brain, 39.7 mM/kg; liver, 40.6 mM/kg; muscle, 39.5 mM/kg) (Fig. 1).

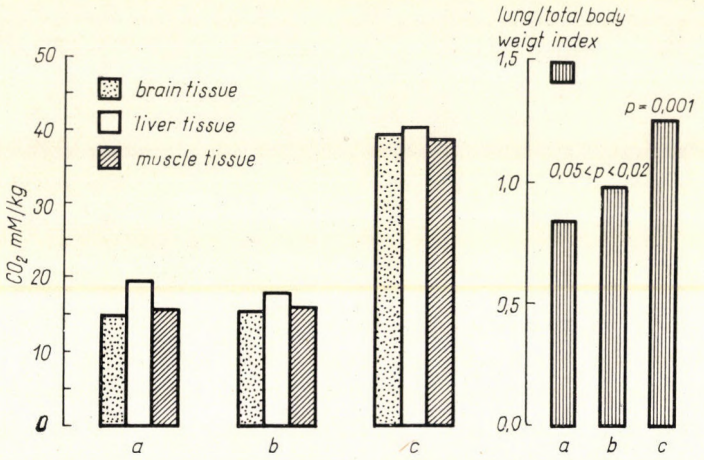

Fig. 1. Tissue CO_2 concentration and lung weight per total body weight index in rats after 2 hrs. exposure to 3 atm. O_2 and to 3 atm. O_2 + CO_2. *a*) Normal control, *b*) Group exposed to 3 atm. O_2. *c*) Group exposed to 3 atm. O_2 + 4 per cent CO_2

Discussion

Laboratory animals breathing high pressure 100 per cent O_2 develop extensive pulmonary oedema and tissue hypercapnia at a rate depending on the applied pressure. Elevation of O_2 pressure shortens survival and accelerates the development of pulmonary changes. MARSHALL and LAMBERTSEN [14] found a similar correlation between pO_2 and survival time in mice.

As to the role of tissue CO_2 content in the induction of pulmonary oedema, hypercapnia attains the level of significance before any pulmonary change has become manifest and before the lung weight index has significantly changed. Among the tissues under study, the earliest evidence of hypercapnia is found in the muscle, next in order is the brain to be followed by the liver. This is at variance with the observation [9] that tissue hypercapnia is confined to the brain. Hypercapnia in muscle tissue is excessive from the very start while in the two other tissues under study it remains slight until the terminal stage when it becomes highly significant. However, in the individual animals no correlation was demonstrable between the degree of tissue hypercapnia and the severity of pulmonary oedema. Histologic evidence of pulmonary oedema may have

been present in certain animals when the CO_2 concentration in the tissues was still normal. In the majority of the animals, however, the pulmonary changes appeared later than the hypercapnia. This fact together with the adverse effect of CO_2 when added to high pressure O_2 suggests that hypercapnia plays a part in hyperoxygenic pulmonary oedema. In respect to O_2 poisoning, similar results have been recorded by BEAN [8], BRENK and JAMIESON [15] and by SZÁM et al. [9]. DOWNING et al. [16] claim that cerebral hypercapnia leads to adrenergic hyperactivity which may well promote the development of pulmonary oedema. Others attribute the adverse effect of CO_2 to the changes in tissue pH, having been able to suppress this effect by the administration of buffers [17, 18]. On the other hand, the absence of any correlation between tissue CO_2 and pulmonary damage pleads against the exclusive role of CO_2 in the induction of hyperoxygenic pulmonary oedema. It is supported by ample evidence that O_2 itself may well account for the pulmonary changes. JAMIESON and BRENK [19] for instance have found in rats exposed to high pressure O_2 a reduced pulmonary dehydrogenase activity preceding the characteristic histologic changes. Pentobarbital-Na, one of the agents affording protection from the effect of hyperoxygenation on the lungs was also found to inhibit the fall in dehydrogenase activity. Antioxidants such as methylene blue, ascorbic acid, alpha-tocopherol-acetate, hydroquinone, etc., have the capacity of prolonging survival [20].

It thus follows from the present study that tissue hypercapnia may play a significant though not exclusive part in pulmonary oedema developing after hyperoxygenation. Closer insight into this mechanism requires further studies.

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STUDIES IN EXPERIMENTAL PULMONARY OEDEMA

II. EFFECT OF DIBENZYLINE ON PULMONARY OEDEMA INDUCED BY HYPEROXYGENATION

By

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The effect of the alpha-receptor blocking agent phenoxybenzamine on the induction of pulmonary oedema by high pressure O_2 was studied.

Previous phenoxybenzamine administration prolonged the survival of animals kept in high pressure O_2 without, however, inhibiting the development of pulmonary oedema.

The slight hypercapnia found in the tissues of the pretreated animals did not contribute to the pulmonary damage.

In phenoxybenzamine-treated rats kept in high pressure O_2 atmosphere, CO_2 concentration did not increase further in the tissues. The adverse effect on pulmonary damage of severe hypercapnia caused by high pressure O_2 + 4 per cent CO_2 was also averted by phenoxybenzamine.

The results suggest that neither tissue hypercapnia nor adrenergic hyperactivity by themselves account for the development of hyperoxygenic pulmonary oedema.

The part played by the adrenergic nervous system in the pathomechanism of hyperoxygenic pulmonary lesion has not failed to attract interest [1, 2]. Adrenalectomy has been found to attenuate [3], epinephrine to aggravate [1] the symptoms of high-pressure O_2 poisoning.

It has been intended by the present experiments to study the effect of the α-adrenergic blocking agent phenoxybenzamine (Dibenzyline, Smith, Kline and French Laboratories, Philadelphia, Pa) on pulmonary oedema due to O_2 poisoning. Since the results of our earlier experiments [4] as well as other data [5, 6] clearly indicate the role of tissue hypercapnia in the production of hyperoxygenic pulmonary lesion, we have studied the changes of CO_2 concentration in the tissues as well.

Method

Seventy five inbred rats weighing between 200 and 300 g were exposed in groups of three animals each to O_2 at a pressure of 3 atm. Continuous absorption of CO_2 on soda-lime was provided for. Survival time was recorded. CO_2 concentration in brain, liver and muscle tissue was determined by the modified ANREP technique [7] after spontaneous death and after 4 hrs. exposure. The severity of pulmonary lesion was assessed by the index lung weight × 100 per total body weight and on histologic evidence.

In one of the groups 4 per cent CO_2 was added to the high pressure O_2. After 2 hrs. exposure the same investigations were carried out as in the former group.

Phenoxybenzamine was administered intraperitoneally in doses of 20 mg/kg 18 hours before the experiment.

Results

Administration of phenoxybenzamine was followed by a significant rise in the CO_2 concentration in the brain, liver, and muscles as opposed to the controls. The lung weight index, however, remained normal (Fig. 1).

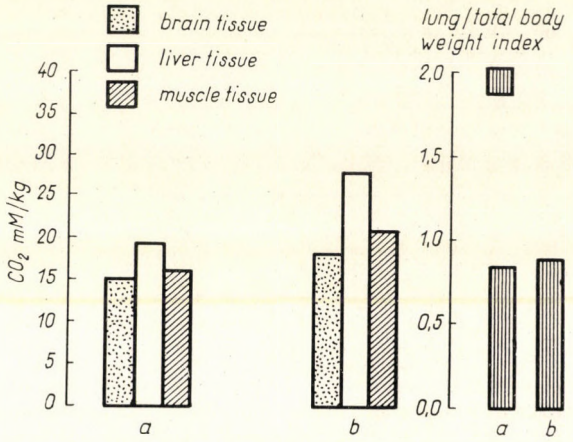

Fig. 1. CO_2 concentration in brain, liver, and muscle tissue and lung weight per total body weight index, in untreated (a) and phenoxybenzamine-pretreated (b) rats

Table I

Effect of 3 atm. O_2 on CO_2 concentration in the tissues, and lung weight per total body weight index in phenoxybenzamine-pretreated rats

Length of exposure	Tissue CO_2; mM/kg			Lung weight/total body weight
	Brain	Liver	Muscle	
Control	18.7±1.29	28.3±1.46	21.8±1.23	0.90±0.04
4 hrs.	20.4±1.90 $p > 0.2$	24.9±2.28 $p > 0.2$	20.5±1.64 $p > 0.8$	0.81±0.04 $p > 0.2$
7 hrs.	20.7±0.87 $p > 0.2$	29.9±1.65 $p > 0.4$	22.4±1.49 $p > 0.7$	1.57±0.06 $p \ll 0.001$

p values, in comparison with phenoxybenzamine-pretreated controls.

The effect of pure O_2 at 3 atm. on tissue CO_2 concentration and the lung weight index in rats pretreated with phenoxybenzamine is shown in Table I. The changes in CO_2 content of the tissues, as compared to those in the controls treated with phenoxybenzamine, were insignificant. After 4 hours exposure, the lung weight index was practically the same as before the experiment, while in

the animals with an average survival of 7 hrs. 18 min. the index reached 1.57, a value indicative of severe pulmonary oedema. In agreement with these figures, the histologic finding at 4 hours was identical with that found in the pre-

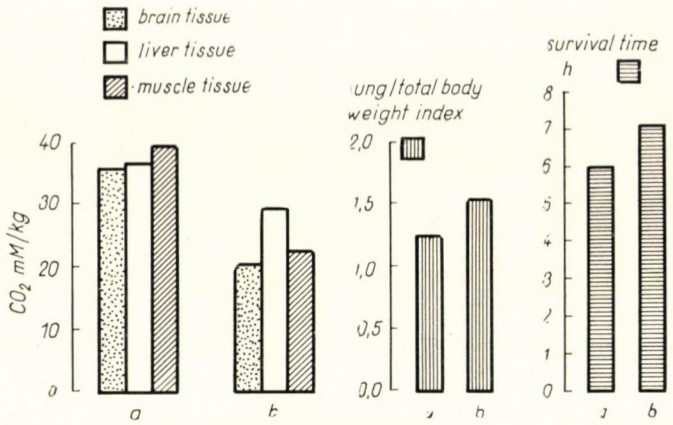

Fig. 2. Survival time, CO_2 concentration in brain, liver, and muscle tissue, and lung weight per total body weight index in untreated (a) and phenoxybenzamine-pretreated (b) rats died on exposure to 3 atm. O_2

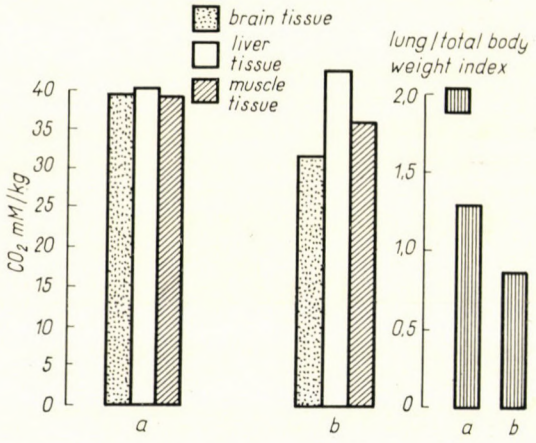

Fig. 3. CO_2 concentration in brain, liver, and muscle tissue, and lung weight per total body weight index, in untreated (a) and phenoxybenzamine-pretreated (a) rats after 2 hrs. exposure to 3 atm. O_2 + 4 per cent CO_2

treated control animals, while in the animals which had died spontaneously there was diffuse alveolar pulmonary oedema.

Comparison with the data for untreated animals (Fig. 2) revealed that survival time at 3 atm. O_2 pressure after phenoxybenzamine pretreatment had increased from the average 6 hrs. 18 min. to 7 hrs. 5 min. ($p < 0.001$). During this time the initial tissue CO_2 concentration had not increased, but pulmonary

oedema was more severe than in the untreated animals. Tissue CO_2 in the rats pretreated with phenoxybenzamine was far from the hypercapnic values of the controls which had been killed by pure O_2 of the same pressure.

Next, the effect of high pressure O_2 + 4 per cent CO_2 was examined. Since by the end of a 2 hour exposure the majority of the animals had succumbed to pulmonary oedema of the severest degree, the animals pretreated with phenoxybenzamine were also kept in the same gas mixture for the same period. Tissue CO_2 started to rise in the untreated as well as in the pretreated animals, to exceed 30 mM/kg in all tissues examined after an exposure of 2 hours (Fig. 3). In the appearance of the lungs there was, however, a substantial difference between the two groups. While the lungs of the untreated animals displayed moderate or gross oedema and the index averaged 1.26, in the pretreated group there was no evidence of pulmonary oedema, and the average index was 0.86.

Discussion

There is ample evidence incriminating the adrenergic system for the pulmonary damage due to high pressure O_2.

It has been shown by GERSCHMANN et al. [3] that adrenalectomy has a beneficial influence on hyperoxygenic pulmonary oedema. These findings have led these authors to assume that high pressure O_2 exerts its toxic influence by enhancing adrenergic activity. This theory is supported by the observation that hyperoxygenic and epinephrine-induced pulmonary oedema have similar features. Investigations by JOHNSON and BEAN [8] into the effect of adrenergic blocking agents revealed that the pulmonary damage was decreased by dibenamine and phenoxybenzamine though the lung weight index was slightly elevated. In these experiments, exposure to high pressure O_2 was, however, discontinued when convulsions had appeared, therefore the results do not reveal conclusively how far the pulmonary damage might have been carried.

In the present experiment survival times and pulmonary changes were compared in untreated and phenoxybenzamine-pretreated rats after exposure to 3 atm. O_2. Prolongation of survival by more than one hour was demonstrable in the pretreated rats but despite the longer survival the pulmonary oedema found at autopsy in these animals was more severe than in the controls.

Blocking of the alpha-receptors thus affords no protection from hyperoxygenic pulmonary oedema, it only delays its onset. Adrenergic hyperactivity is possibly only one of the factors of pulmonary damage. The effect of catecholamines to cause pulmonary oedema is a well documented fact. In crossed circulation experiments we have shown that epinephrine exerts this effect through the agency of the central nervous system [9]. Epinephrine-induced pulmonary oedema was fully inhibited by previous phenoxybenzamine administration [10].

According to JOHNSON and BEAN [8] increased adrenergic activity is mediated by the central nervous system, possibly as a result of cerebral hypercapnia. SZÁM et al. [6] also ascribe a part to cerebral hypercapnia in the production of hyperoxygenic pulmonary oedema.

On the evidence of our findings, the CO_2 concentration in brain, liver, and muscles was higher in phenoxybenzamine-pretreated rats than in the controls without, however, being sufficient by itself to induce pulmonary oedema. As opposed to untreated animals, tissue CO_2 in phenoxybenzamine-treated animals did not increase further on exposure to high pressure O_2. Accumulation of CO_2 in the tissues in consequence of hyperoxygenation was thus averted by phenoxybenzamine pretreatment probably through an increase of the blood supply or through metabolic changes.

Excessive hypercapnia in the tissues due to the presence of CO_2 in high pressure O_2 was demonstrable in the treated and untreated animals alike, as a consequence of the high pCO_2 in the inhaled gas mixture, but no aggravation of the pulmonary lesion by CO_2 was noted in the pretreated rats. In our earlier experiments we found no evidence of hypercapnia or of pulmonary lesion in untreated animals after exposure for 2 hours to 3 atm. O_2 [4]. On the addition of 4 per cent CO_2 to the high pressure O_2, pulmonary oedema was found in all the animals after an exposure of 2 hours and 3 out of 10 rats had died by this time. On the other hand, all the pretreated animals survived and developed no pulmonary oedema even when 4 per cent CO_2 had been added to the high pressure O_2.

The present results seem to suggest that adrenergic hyperactivity may contribute to the severity of hyperoxygenic pulmonary damage, and the mechanism may be connected with hypercapnia. Hypercapnia was found not only in the brain but also in the other examined tissues. But neither of these factors alone, i.e. adrenergic hyperactivity, or hypercapnia, do account for the induction of the pulmonary oedema. This clearly follows from the observation that previous phenoxybenzamine administration inhibited the accumulation of CO_2 in the tissues but not the induction of pulmonary oedema. No aggravation of pulmonary damage by exogenous hypercapnia was, however, demonstrable in the phenoxybenzamine-pretreated rats.

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STUDIES IN EXPERIMENTAL PULMONARY OEDEMA

III. EFFECT OF ADRENOLYTIC DRUGS ON EPINEPHRINE-INDUCED PULMONARY OEDEMA IN THE RAT

By

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Epinephrine injected intravenously caused convulsions followed by lethal pulmonary oedema in 100 per cent of the rats. Previous administration of dichlorisoproterenol failed to modify the course of events, while phenoxybenzamine fully inhibited both the convulsions and pulmonary oedema and all the animals were alive and well 48 hours after the otherwise lethal injection of epinephrine.

In crossed circulation experiments we have shown that epinephrine-induced pulmonary oedema is neurohaemodynamic in origin, being the outcome of circulatory changes mediated by the central nervous system [1]. It is, however, not clear whether the catecholamines caused the haemodynamic changes responsible for pulmonary oedema by affecting the alpha- or beta-receptors of the heart or the other organs. In the present study we have sought an answer to that question.

Material and method

Seventy eight male inbred rats weighing between 140 and 240 g were used. Pulmonary oedema was induced with 1 mg/kg epinephrine by the intravenous route without previous treatment and after previous injection of dichlorisoproterenol (EGA-Chemie K. G., Keppler und Reif) and phenoxybenzamine (dibenzyline, Smith, Kline and French Laboratories, Philadelphia, Pa). Dichlorisoproterenol was given in an intravenous dose of 5 mg/kg 15 to 30 minutes before the injection of epinephrine. Phenoxybenzamine was given intraperitoneally in a dose of 10 mg/kg 20 to 24 hours before the injection of epinephrine. Untreated, dichlorisoproterenol and phenoxybenzamine-treated animals were used as controls.

The animals were killed by decapitation without anaesthesia at the end of the experiment.

We recorded the time of onset of the convulsions, the survival time and the pulmonary changes. The presence of pulmonary oedema was inferred from the lung weight $\times$ 100 per total body weight index as well as from the gross and microscopic appearance of the lungs.

Results

Table I shows the results and Fig. 1 the lung weight indexes.

The lung weight index in the untreated control group averaged 0.77, in the dichlorisoproterenol-treated controls 0.86, in the phenoxybenzamine-treat-

ed controls 0.92; hardly deviating from normal. One to four minutes (mean, 1.5 min.) after the injection of epinephrine, tonic-clonic convulsions appeared in the untreated animals and death ensued in 4 to 10 minutes (mean, 6 min.). There were no survivors. The lung weight index averaged 2.04, the lowest value was 1.55. These figures were consistent with the gross pulmonary changes (dark red colour, swelling, oedema fluid filling the bronchi). Pulmonary oedema was demonstrable also histologically.

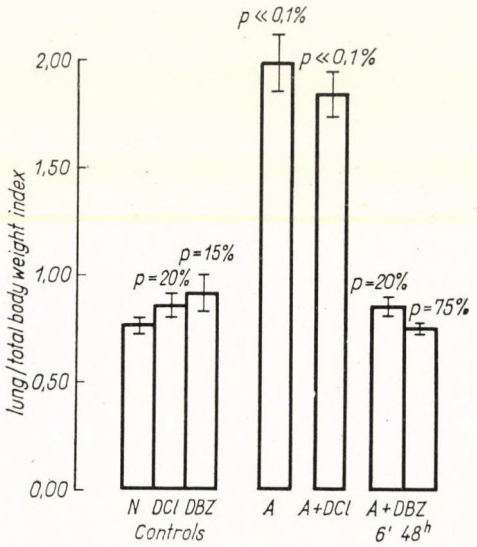

Fig. 1. Lung weight per total body weight index in rats: normal; treated with dichlorisoproterenol; treated with phenoxybenzamine, with epinephrine, with epinephrine + dichlorisoproterenol and with epinephrine + phenoxybenzamine

In the animals pretreated with dichlorisoproterenol, convulsions and pulmonary oedema released by epinephrine were of the same intensity as in the controls. The convulsions appeared 1.5 minutes after the epinephrine injection and average survival was 5.6 minutes. The gross appearance of the lungs were similar to those of the animals which had received epinephrine only. The average index value was 1.85.

All the animals pretreated with phenoxybenzamine survived the dose of epinephrine, and exhibited no convulsions. In half of the animals, sacrificed at 48 hours, the lungs were normal, and the index averaged 0.76, i.e. it was in the normal range. To determine whether in the phenoxybenzamine-pretreated animals epinephrine caused transitory pulmonary changes, the other half of the animals in this group was killed at the time when the animals which had received no phenoxybenzamine, died. Here, too, the lungs were normal, the index averaged 0.80, showing no significant divergence from normal.

Table I

Effect of pretreatment with dichlorisoproterenol and phenoxybenzamine on epinephrine-induced pulmonary oedema in the rat

	No. of animals	Time (minutes) of		No. of survivors	Lung weight/total body weight	p-values correlated to normal, %
		convulsion	spontaneous death			
Normal controls	12	—	—	12	0.77 ± 0.04	
Dichlorisoproterenol-treated controls	6	—	—	6	0.86 ± 0.06	20
Phenoxybenzamine-treated controls	6	—	—	6	0.92 ± 0.09	15
Epinephrine	12	1.5	6.1	—	2.04 ± 0.14	⩽ 0.1
Epinephrine + dichlorisoproterenol	12	1.5	5.6	—	1.85 ± 0.11	⩽ 0.1
Epinephrine + phenoxybenzamine (6 minutes)	12	—	—	12	0.80 ± 0.05	20
Epinephrine + phenoxybenzamine (48 hours)	12	—	—	12	0.76 ± 0.03	75

Discussion

The effect of epinephrine is known to assert itself through two sympathetic receptor types, i.e. alpha-receptors responsible for the excitatory responses (smooth muscle contractions) with the exception of the heart, and beta-receptors mediating the positive chrono- and inotropic responses of the heart but being inhibitory (i.e. vasodilative) in other organs [2, 3, 4]. In the present experiments neither dichlorisoproterenol nor phenoxybenzamine alone caused any change in the weight or in the histologic structure of the lungs.

On the evidence of the literature as well as of our own studies [1] the first link in the chain of events through which epinephrine leads to pulmonary oedema is vasoconstriction as a response to sympathetic excitation in consequence of which a considerable part of the circulating blood is diverted from the peripheral circulation to the lungs. This places an added load upon the lesser circulation.

In the present studies it was not possible to inhibit the development of epinephrine-induced pulmonary oedema or to influence survival by previous administration of dichlorisoproterenol. This was only to be expected considering the haemodynamic effects of the two drugs. Dichlorisoproterenol poten-

tiates the vasoconstrictive effect of epinephrine and in doses as small as 3 mg/kg it completely blocks the positive chrono- and inotropic effects in the heart [4].

In contrast, full inhibition of pulmonary oedema was achieved by phenoxybenzamine administered prior to epinephrine. Convulsions remained absent and 48 hours after the administration of epinephrine all animals were in good health. Not even transitory changes were present in the lungs at a point of time when all the animals which had been given epinephrine alone had succumbed to pulmonary oedema.

Phenoxybenzamine does not affect the sympathetic innervation of the heart [2] while blocking the alpha-receptors of other organs. In this manner it is not by a direct cardiac action that it averts the pulmonary oedema but rather by blocking the vasoconstrictor response, and by this fact also the increase in peripheral resistance or, as another alternative, by relieving the spasm of the pulmonary vessels and thus reducing pulmonary filtration pressure. The close connection in time between the onset of tonic-clonic convulsions and the appearance of pulmonary oedema illustrates the importance of the neurogenic factor. Our observations in this respect agree with those of JOHNSON and BEAN [5]. SZÁM et al. [6] have been led to similar conclusions by electroencephalographic evidence. By previous administration of phenoxybenzamine not only the development of pulmonary oedema but also that of the convulsions was inhibited. This makes it likely that the effect of phenoxybenzamine involves the central nervous sites of action of epinephrine.

It has been shown [7] that in experimental shock, a condition also associated with adrenalinaemia, cerebral O_2 demand is increased. This is, however, not the case if phenoxybenzamine has been administered previously. The protection from epinephrine-induced pulmonary oedema conferred by phenoxybenzamine may originate in a similar mechanism involving the metabolism of the brain.

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19—20 (HEREDITARY?) TRISOMY IN A FAMILY WITH MULTIPLE CONGENITAL MALFORMATIONS

By

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A family is described in which through four generations several members had congenital malformations, mostly polycystic kidney (?) and congenital dislocation of the hip. Chromosomal examination was carried out in five members. The patient with multiple congenital malformations and her tall, but otherwise healthy daughter had 46/47 mosaic F-trisomy.

Supernumerary or lacking sex chromosomes may give rise to well-known clinical pictures (syndromes of KLINEFELTER and TURNER), though the XXX female (superfemale) [1] and the XYY male [24] may be phenotypically quite normal. The autosomal deletions or trisomies have, however, less circumscribed clinical patterns. The most characteristic among them are the 21, 13, 18 trisomies and 5 partial monosomy [2—4]. The clinical findings in trisomies of other autosomes of groups A, C, F and G are not characteristic [5—16]. Our observation belongs to the latter, clinically indefinite group of autosomal trisomies.

Case report

A. S. 36-year-old female (IV/1 on Fig. 1) was admitted on 28th September 1962. She had been born 3 months before term, with bilateral dislocation of the hip. In her family there were in four generations (see the family-tree in Fig. 1) 7 cases of congenital dislocation of the hip (I/1, II/1, IV/1, 2, 7, V/6, 7), 6 of "renal disease" with fatal outcome (III/1, 3, IV/3, 4, 5,7), 9 other congenital malformations (I/1, III/2, 4, IV/1, 5, V/2, 3, 4, 5) and 1 chronic myeloid leukaemia (III/4). Menarche at 16 years, 5 pregnancies resulted in 3 live born children, 1 ectopic pregnancy and 1 arteficial abortion.

The patient had had spastic epigastrial pains for two years before admission and felt a slightly painful tumour in the left part of the abdomen. Some months ago she had become aware of a tenderness in the back, on the right side of the spine. She had lost 2 kg weight during the last 2 months.

Physical examination and laboratory findings

Poorly developed, thin female. Gait characteristic of bilateral dislocation of the hip. There are no nails on fingers or toes except the little finger of the right hand (Fig. 2). Deformed thorax due to scoliosis; dermoid cyst in the right lumbosacral region. Blood-pressure, 130/90 mmHg. Palpable kidneys, shifted towards the midline, with irregular surface. NPN, creatinine clearance and serum-electrolytes normal. Retrograde urography and palpation revealed polycystic kidneys bilaterally. Paretic external rectus muscle of left eye; it causes double vision on the left side. Normal intelligence.

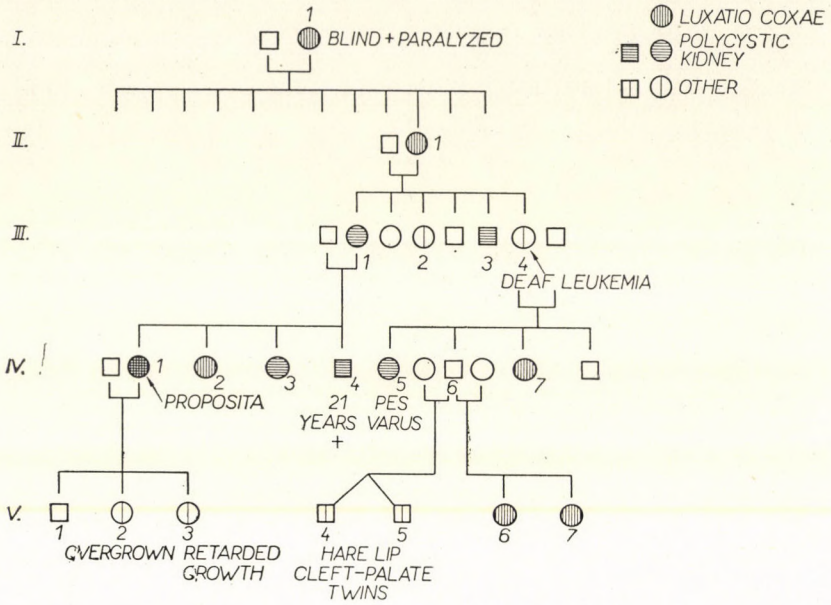

Fig. 1

Table I

Chromosome number	42	43	44	45	46	47	Total
A. S. (female) IV/1.	3	2	1	3	20	21	50
A. S. (female) IV/2.				1	18	1	20
A. S. (male) V/1.		1		3	14	2	20
A. S. jr (female) V/2.			3	5	42	27	77
J. S. (female) V/3.					15		15

Chromosomal examination

The chromosomal examination was made from the peripheral blood culture by Moorhead's method slightly modified [17]. 50 cells were scored; 20 cells contained 46 chromosomes and 21 cells contained 47 chromosomes (Table I). The 47th chromosome seemed to belong to group F (Fig. 3). The peripheral blood culture revealed 8 per cent sex-chromatin-positive cells.

A. S. (V/2), daughter of the former patient, aged 13 years. She is tall (177 cm) for her age, but otherwise normal. From the 77 cells scored from the peripheral blood culture, 42 cells had 46 chromosomes and 27 cells had 47 chromosomes (Table I). The 47th chromosome was similar to those of group F (Fig. 4). Sex-chromatin was positive (10 per cent; peripheral blood culture).

J. S. (V/3), 12-year-old girl, sister of the former patient. Normal development till the age of 6 years; since then she had lagged in growth (present height 135 cm), as compared with her sister (Fig. 5). Chromosomal analysis from the peripheral blood culture showed no abnormality.

A. S. (V/1), son of the first patient. No abnormality, normal karyotype.

A. S. (IV/2), 35 years old, sister of the first patient. She was prematurely born and has bilateral dislocation of the hip. No other abnormalities, normal karyotype.

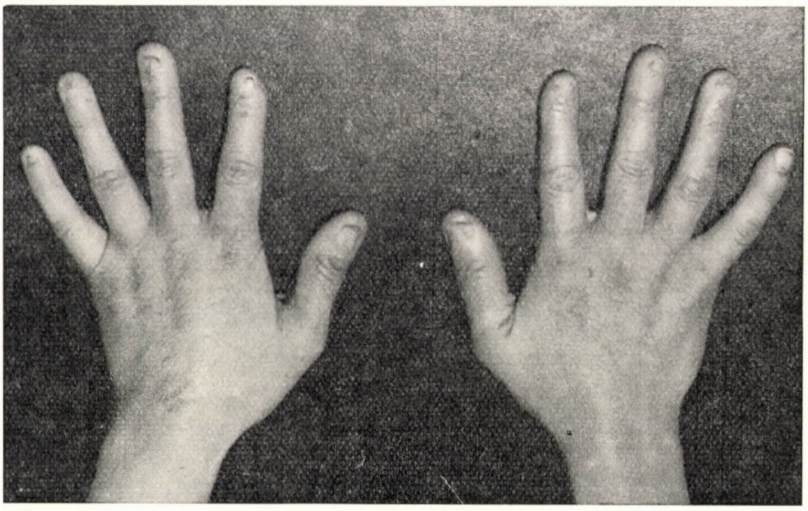

Fig. 2

Discussion

In 4 generations of the family under study there were members with congenital malformations. The most frequent anomalies were dislocation of the hip and renal disease. The latter was probably polycystic kidney, as according to the relatives all those persons had died with uraemia without having had previous signs of acute nephritis. Chromosomal analysis showed in the patient and her tall but otherwise healthy daughter a 46/47 mosaicism; the 47th chromosome seemed to belong to group F. There are of course other possibilities, and the accessory chromosome may have been a chromosome 17 or 18 with delated long arms, or even a delated X chromosome in spite of the single sexchromatin positivity. It could be taken also for an isochromosome of the short arms of chromosome 17 or 18, but the lack of the syndrome characteristic in trisomy 18 did not support this suggestion. The other, healthy daughter, the son, and the sister of the proposita presented no chromosomal aberration.

There are few data concerning F trisomy. BÖÖK et al. [8] found it in a mother and son, both afflicted with an atrial septal defect. GUSTAVSON et al. [9] observed the same trisomy in a case with multiple congenital malformations (microcephaly, mental retardation, epilepsy). The case of CRAWFORD [20] probably also belonged to this group of autosomal trisomy. FRACCARO et al. [7] found F trisomy in a healthy male, but the child of this apparently healthy man had mongoloid cretinism.

In the family under study congenital malformations were frequent and the question arose, what role the observed chromosomal aberrations had in the

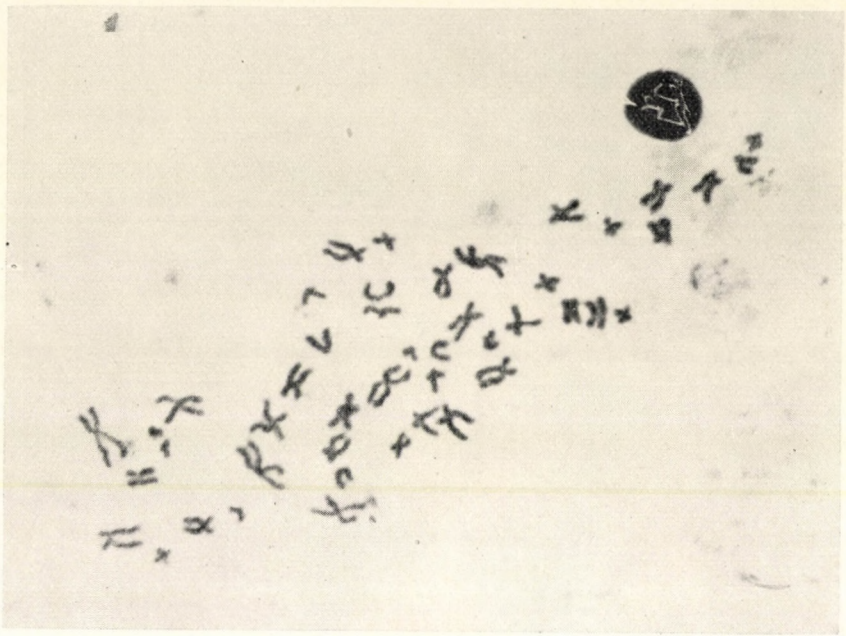

Fig. 3a

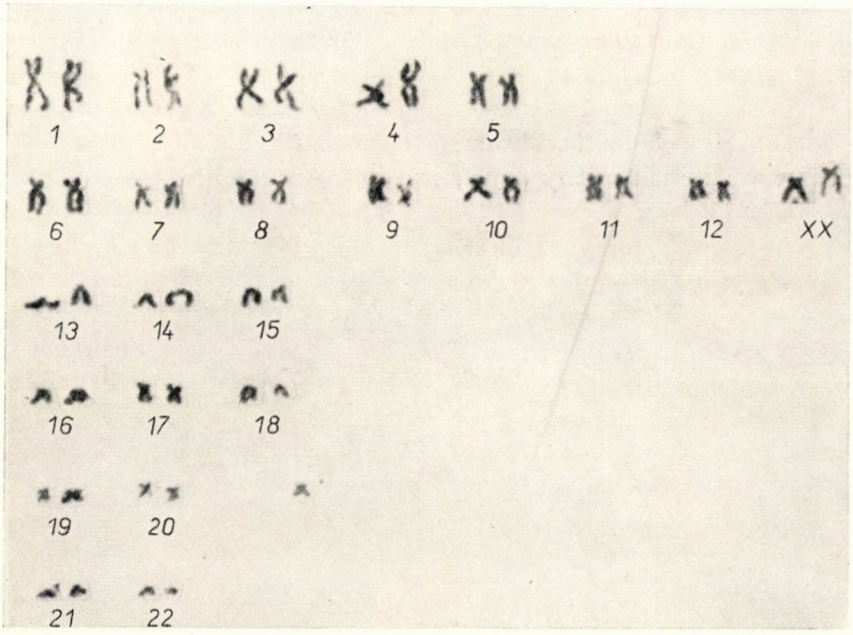

Fig. 3b

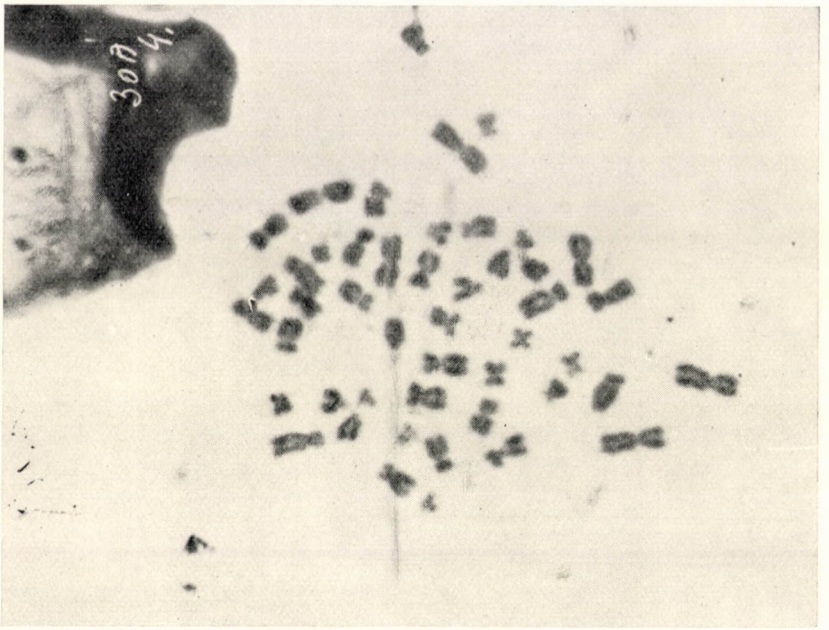

Fig. 4a

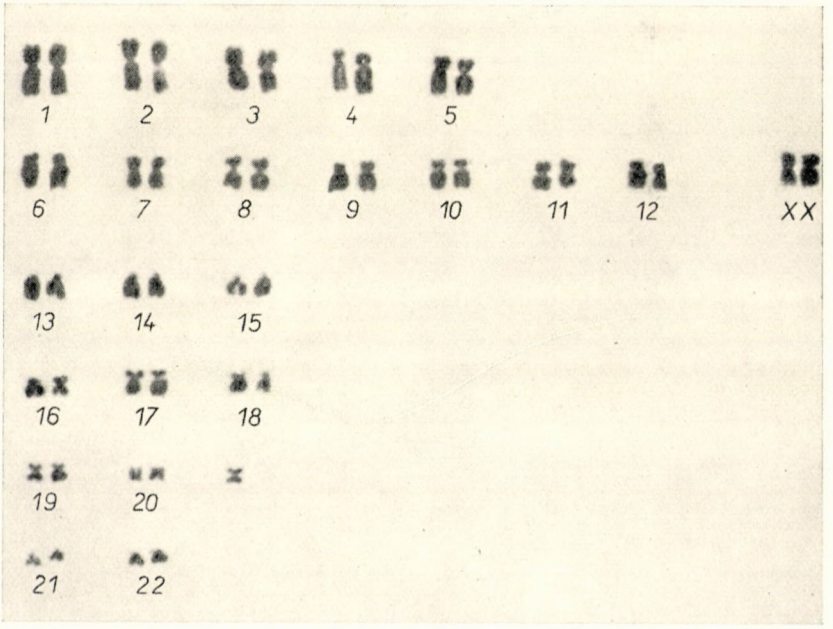

Fig. 4b

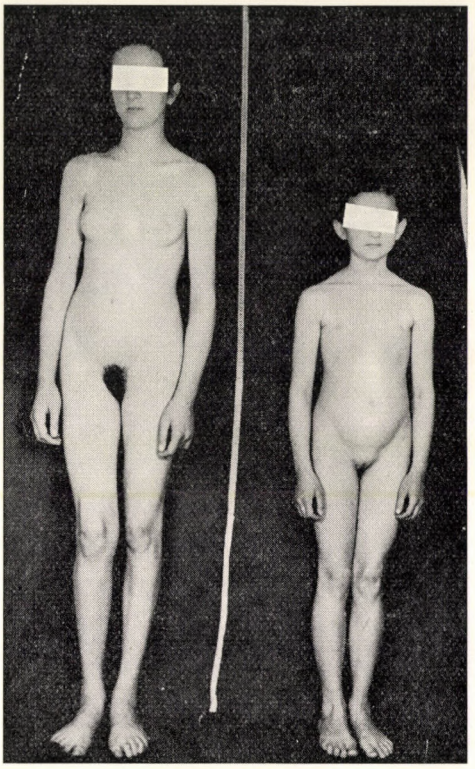

Fig. 5

aetiology of the malformations. The observation, that not all the persons in the family had the same type of congenital malformation, does not exclude their role. (The trait may be a not sex-linked, dominant character with variable expressivity, or the "unbalanced" genom had manifested itself with various clinical patterns). It is not clear why the anomaly was present in a mosaic form in the daughter instead of a normal karyotype or a simple trisomy, without mosaicism, though HAUSCHKA et al. [24], THERMAN et al. [25], TURNER et al. [26] suppose a genetical predisposition to non-disjunction in familial trisomy with different karyotypes. There have been reports on the familial occurrence of mosaicism and it was assumed that this represented a genetically determined tendency to mosaicism [27, 28, 29, 30]. Our case might, therefore, have been either a remarkable coincidence (*vide* the recent suggestions of COURT BROWN [31] concerning chromosomal variations in the "normal" population), or an example supporting the hypothesis of the mentioned authors in that the double incidence had a hereditary character to non-disjunction.

In the patient's daughter, in whom the chromosomal anomaly was revealed, no malformation could be detected. There are observations, where chromo-

somal aberrations of the number [1, 7, 20, 23, 24] or of the shape [19, 22, 31] were associated with a normal phenotype (sometimes through two generations). In some of these cases the same chromosomal abnormality was present not only in the apparently healthy member of the family but, similarly as in our patients, also in a member displaying congenital malformations [20, 21, 24].

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PREVENTION AND TREATMENT OF SHOCK WITH CORTICOSTEROIDS

EFFECT OF PREDNISOLONE IN NOREPINEPHRINE- AND EPINEPHRINE-INDUCED SHOCK

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Phenoxybenzamine in doses of 0.5 mg/kg has been found to exert a protective effect in irreversible shock induced by norepinephrine infusions in dogs. 50 per cent of the animals survived. Prednisolone in doses of 10 to 15 mg/kg failed to protect from shock induced by epinephrine or norepinephrine and even reduced survival time to 50 per cent and enhanced the pressor and haemoconcentrative effects of norepinephrine. On the grounds of the results it seems unjustified to link up the alleged shock-protective effect of corticosteroids with a blockade of the adrenergic endings.

WEIL et al. reported in 1940 that shock mortality in rabbits was considerably reduced by the combined administration of DOCA and adrenocortical extracts. During the following years conflicting data were published concerning the subject. In recent years, however, it has been suggested more and more clearly by the results of clinical and experimental studies that massive doses of glucocorticoids, particularly dexamethasone, prednisolone or hydrocortisone, afforded protection from experimental or clinical shock [2, 3, 4]. The mechanism of the effect is not fully understood. Though in the majority of laboratory animals the critical fall of blood pressure could be averted and survival attained, nevertheless no effect on cardiac output and peripheral resistance was demonstrable [5].

The possibility of an adrenergic blocking effect of corticosteroids similar to that of phenoxybenzamine, was pointed out by certain authors [6]. We believe, however, that the glucocorticoids, on the contrary sensitize the adrenergic nerve endings to the effect of endogenous and exogenous catecholamines. In order to clarify the question, we have studied the effect of pharmacological doses of glucocorticoid (prednisolone) on lethal shock induced by norepinephrine and epinephrine.

Methods

Mongrel dogs of both sexes were studied under intravenous pentobarbital (30 mg/kg) anaesthesia. Shock was induced by 1.7 mg/kg of norepinephrine or epinephrine, given in 8 ml/kg of physiological saline as an intravenous drip, at an even rate over three hours. Five groups of 15 animals were studied. Group I was given a norepinephrine infusion alone. Group II received 10 to 15 mg/kg prednisolone intravenously one half hour before, and the same dose at the start,

of the norepinephrine infusion. Group III was given 0.5 mg/kg of phenoxybenzamine intravenously half an hour before the infusion of epinephrine. The animals to be compared were matched in a manner to be approximately of the same body weight in each group. The animals of Group IV were given an infusion of epinephrine, those of Group V received 15 mg/kg of prednisolone half an hour before and the same amount at the start of infusion. Here, too, one animal which received epinephrine alone was matched with another which received epinephrine + prednisolone.

As soon as the infusion had been completed, the animals were placed in a cage. Animals which were found alive 24 hours after completion of the infuson and came out of anaesthesia moving about briskly in the cage were considered survivors.

Arterial blood pressure was measured during the infusion by means of a mercury manometer inserted into the femoral artery. The haematocrit ratio and plasma protein concentration were determined from blood samples taken every hour.

Results

In Groups I and II all of the animals died. Mean survival time was 13.47 (SD 7.40) hours in Group I, treated with norepinephrine alone. In Group II, treated with norepinephrine and prednisolone, survival was shorter, with a mean of 7.75 (SD 7.76) hours. The difference was significant ($p < 0.05$). In Group III 7 out of the 15 animals which had been given phenoxybenzamine prior to norepinephrine survived over 24 hours. The 8 animals which died had an average survival of 14.27 (SD 7.98) hours. The animals with epinephrine infusion (Group IV) also died, mean survival was 10.76 (SD 8.6) hours. Survival in Group V was shortened by prednisolone to 5.34 (SD 5.59) hours. The difference was significant ($p < 0.05$).

The pressor effect of norepinephrine or epinephrine reached the peak at the beginning of the infusion. Subsequently, arterial pressure continually declined despite the infusion until it was well below the initial value. Half an hour after the start of infusion in Group I (norepinephrine) blood pressure was 17 ± 5.1 mm Hg, in Group II (norepinephrine + prednisolone) 32 ± 7.8 mm Hg higher than the initial values. The difference between the two groups was significant. Epinephrine infusion (Group IV) was found to increase blood pressure by 29 ± 4.8, and epinephrine + prednisolone (Group V) to the same extent, i.e. by 29 ± 5.6 mm Hg. Phenoxybenzamine (Group III) was found to inhibit the pressor effect of epinephrine: half an hour after the start of epinephrine infusion, blood pressure was 3 ± 11.1 mm Hg lower than the initial value.

Prednisolone appeared to enhance haemoconcentration due to norepinephrine. The haematocrit was at its peak two hours after the start of infusion, the initial mean of 39.1 (SD 5.0) found in Group I before infusion having reached 50.4 (SD 10.1). In the prednisolone-treated animals (Group II) the mean of 41.6 (SD 8.0) rose to 55.3 (SD 6.8). The difference in the increases of haematocrit ratios was significant statistically ($p < 0.05$). Phenoxybenzamine had a preventive effect on haemoconcentration due to norepinephrine, the initial mean haematocrit value of 43.7 (SD 6.0) in Group III declined to 43.5 (SD 6.5)

Effect of phenoxybenzamine and prednisolone on survival, blood pressure and haematocrit, in shock induced by norepinephrine and epinephrine

		n	Survival, hours	Blood pressure mm Hg					Haematocrit				
				before	0.5	1	2	3	before	0.5	1	2	3
Norepinephrine	M	15	13.47	150	165	144	126	100	39.1	47.8	49.3	50.4	48.9
	SD		7.42	42	26	38	42	42	5.0	8.1	10.1	10.1	7.5
Norepinephrine + prednisolone	M	15	7.75	150	185	149	119	112	41.6	49.9	51.4	55.3	54.6
	SD		7.76	22	23	26	34	34	8.0	6.7	8.2	6.8	6.0
Norepinephrine + phenoxybenzamine	M	15	*14.29	138	133	120	100	84	43.7	46.2	43.8	43.5	44.3
	SD		7.98	32	32	32	25	31	6.0	2.8	5.4	6.5	6.3
Epinephrine	M	15	10.76	156	188	167	129	85	49.3	54.1	56.4	55.8	52.7
	SD		8.26	19.1	13.45	21.2	24.7	43.2	7.1	10.9	6.9	6.4	4.3
Epinephrine + prednisolone	M	15	5.34	165	194	173	113	67	45.9	51.3	52.9	53.4	51.4
	SD		5.59	18.0	25.3	20.5	35.5	30.0	8.7	9.8	10.8	11.3	7.6

* The figures refer only to those animals which died.

within two hours after the start of the infusion. In contrast, prednisolone had virtually no effect on haemoconcentration due to epinephrine, a rise from 49.3 (SD 7.1) to 55.8 (SD 6.4), corresponding to an average of 6.5 per cent, was found in Group IV, whereas in Group V a rise from 45.9 (SD 8.7) to 53.4 (SD 11.3), or 7.5 per cent was demonstrable. The difference between Group IV and Group V was not significant.

Discussion

Phenoxybenzamine in doses of 0.5 mg/kg had a significant protective effect on the irreversible shock induced by massive doses of epinephrine. Around 50 per cent of the animals survived, and remained free from the pressor and haemoconcentrative effects of norepinephrine. These findings agree with those of FITTS and SAWYER [7]. In contrast, prednisolone failed to influence the shock induced by epinephrine or norepinephrine and even enhanced the sensitivity to catecholamines, as suggested by the significant reduction of survival as well as by the increase of the pressor response and haemoconcentration released by norepinephrine.

The present observations thus plead against the view ascribing the protective effect of corticosteroids in experimental endotoxin [2], ischaemic [3] or haemorrhagic [4] shock to a sympathetic blockade similar to the effect of phenoxybenzamine. Our findings are at variance with those of LILLEHEI et al. [6] on which these authors have based the claim that hydrocortisone protects from epinephrine-induced shock.

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POSTISCHAEMIC RENAL FAILURE IN UNANAESTHETIZED DOGS

By

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1. In 13 dogs renal ischaemia was induced by clamping the artery for two hours. The procedure was carried out on unanaesthetized animals, causing no pain. Ten animals died with signs of uraemia, 3 animals survived. The difference was significant statistically.

2. Laboratory results and histological findings suggested the identity of human disease and the produced type of experimental renal failure.

3. On the basis of the present and previous experiments it is assumed that the mechanism of the experimental renal failure is a reflex process which might perhaps explain the development of human acute renal failure.

Temporary occlusion of the renal artery in animals induces a postischaemic disturbance of the kidney which in many respects is similar to human acute renal failure (BADENOCH [1], DUTZ and KRETZSCHMAR [13], GÖMÖRI and NAGY [17], HAMILTON, PHILLIPS and HILLER [18], KOLETSKY and GUSTAFSON [19], PHILLIPS and HAMILTON [22]). Most authors have suggested that the duration of clamping of the renal artery should last 4 to 6 hours to produce postischaemic changes. SELKURT [25, 26] as well as HAMILTON and PHILLIPS [18, 22] have found only temporary changes of renal function if the duration of ischaemia did not exceed 2 hours. In a previous paper [4] it has been shown that renal failure fails to develop if occlusion of the renal artery has been maintained for two hours under deep chloralose anaesthesia. In contrast, postischaemic renal failure developed if the animals had been anaesthetized superficially with morphine and ether. The fact that animals under deep anaesthesia survived the 2 hours renal ischaemia proves that this condition cannot be responsible for the renal failure. It proves at the same time the prominent role of the central nervous system in the development of renal failure under superficial anaesthesia. This assumption has been supported by the observation that sectioning of the splanchnic nerve or treatment with chlorpromazine prevents renal failure even in superficially anaesthetized animals (FEKETE, TARABA, VISY [15], TARABA [31]). SHEEHAN [28] published similar results for the rabbit.

The phenomenon has been attributed to the active participation of the central nervous system, causing a disturbance in circulation. Deep anaesthesia diminishing the influence of the nervous system, prevented major changes in renal function or, else, morphine may have a noxious effect on renal circulation

or function. It is also possible that the animals under superficial anaesthesia had to suffer too much pain during the operation. In order to throw light on this question, a method has been elaborated to induce postischaemic renal failure in the unanaesthetized dog.

Methods

Female mongrel dogs were divided in two groups. In the first group of 8 dogs perineotomy and right nephrectomy were performed. In the second group containing 5 dogs simultaneously with right nephrectomy the left ureter was sewed to the abdominal wall. Two weeks later the left kidney was exposed by a lumbar incision, the renal artery was exposed and put in a loop of plastic thread. The thread was pulled into a plastic tube, which was fixed to the muscles, leaving about a 5 cm long part of it outside the skin. The wound was closed in a sterile way. Four days later the thread was pulled tight for 2 hours, to cause complete renal ischaemia. In this position the renal artery lay in the loop of the thread and circulation ceased in it. Occlusion of the renal artery was controlled by measuring urine flow in a funnel fixed on the abdominal wall, or by catheterizing the dogs before and after the occlusion.

After death or sacrificing the animals the kidneys were removed, embedded in paraffin, sections 6 μ thick were made and stained with haematoxylin-eosin.

Blood samples were taken the first day after the ischaemia and thereafter every second day, to determine the non-protein nitrogen level (CLEGHORN and JENDRASSIK [11]). Serum and urine sodium and potassium levels were determined with flame photometry (Zeiss). Osmolality of plasma and urine was determined by an Advanced Instruments osmometer.

Urine was collected in the first group of animals kept in a metabolic cage for 24 hour periods. In the second group where the ureter was sewed to the abdominal wall urine was collected every second day three times at 20 minutes interval, in a Pavlov stand. The creatinine contents of plasma and urine were determined by BROD's method and expressed in mg per 100 ml. Creatinine clearance was computed for sq.m. body surface.

Statistical evaluation was carried out by the χ^2 test as described by FISHER [16].

Results

The animals alive 14 days after clamping were considered to have survived. Of the 13 animals, 10 died on the 5—9th days. There was a statistically significant difference between the deeply anaesthetized und unanaesthetized groups with respect to mortality ($p < 0.01$). The NPN level (Fig. 1) showed

Table I

Effects of clamping the renal artery

	Survival	Spontaneous death	No. of cases
Superficial morphine-ether anaesthesia*	4	10	14
deep chloralose anaesthesia*	16	1	17
unanaesthetized	3	10	13

Data are from BÁLINT, FEKETE and TARABA [4].

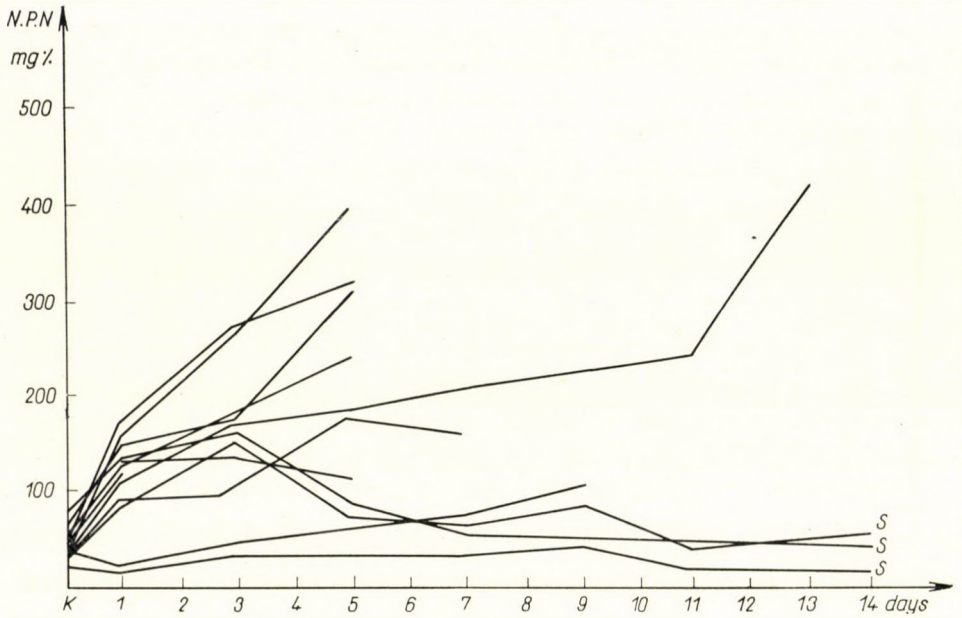

Fig. 1. Serum non-protein-nitrogen level after clamping of the renal artery without anaesthesia. *K* represents the values prior to clamping. *S* represents levels of animals which survived the 14th day after clamping

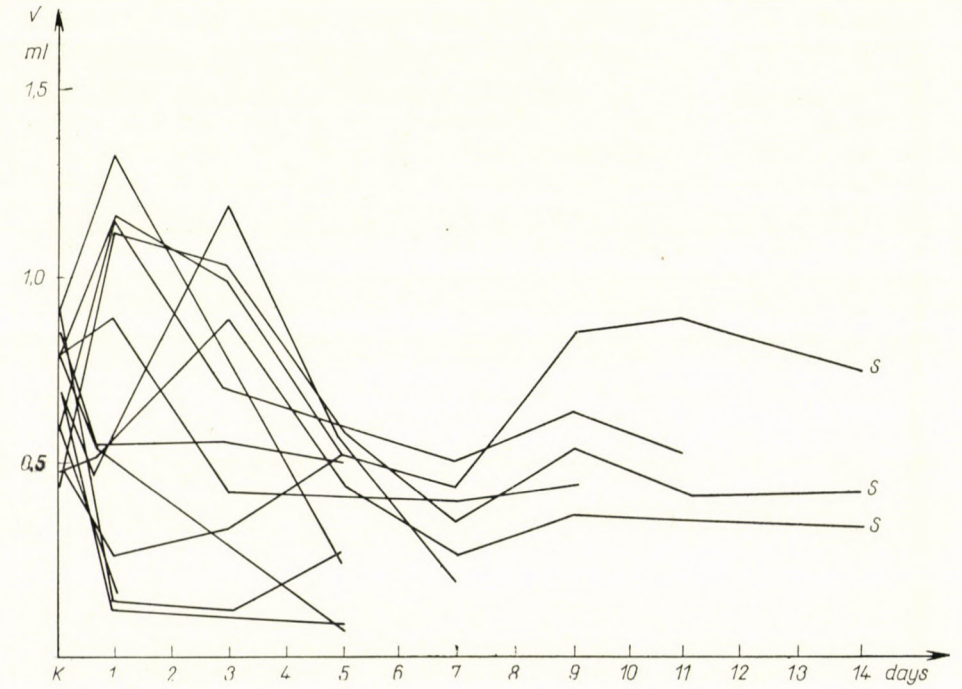

Fig. 2. Urine flow, computed for sq.m. Signs as in Fig. 1

an increase in the first three days and then the animals soon died. Three animals whose NPN level did not rise so rapidly died with a 100 mg per 100 ml NPN level.

The surviving animals showed a temporary increase in NPN which approached the normal value at the end of the second week.

Figure 2 illustrates urine flow per minute and Fig. 3 the endogenous creatinine clearance values.

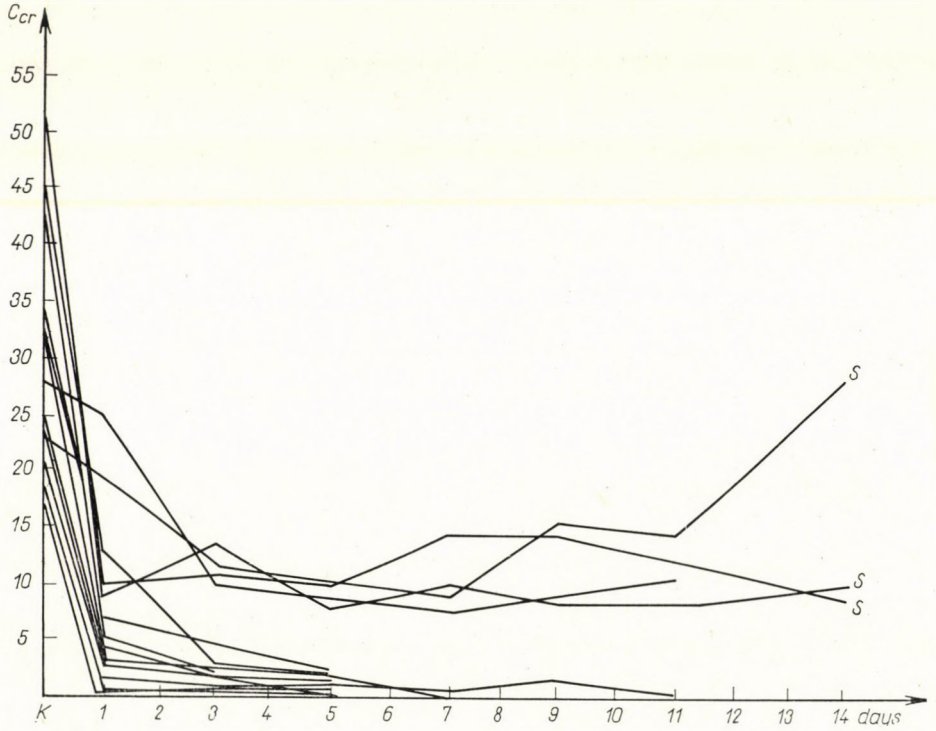

Fig. 3. Endogenous creatinine clearance computed for sq.m. body surface. Signs as in Fig. 1

Urine flow in some animals decreased after clamping the renal artery. The oliguric phase was shortly followed by anuria. Some animals, on the other hand, showed a transitory polyuria. Subsequently either anuria set in or diuresis approached the normal level. The decrease of creatinine clearance was indicative of a diminution of glomerular filtration rate. Most animals had very low clearances.

Tubular concentrating capacity is characterized by the osmolal U/P ratio. This value showed in most animals a considerable decrease, while the survivors with one exception yielded values similar to those of the controls (Fig. 4).

Figure 5 illustrates the serum potassium values. The animals had potassium levels of 6 to 9 mEq per litre.

The morphological changes in the kidney and the severity of the clinical picture showed an approximate correlation, in agreement with data in the literature. In general, the kidneys of the spontaneously died animals displayed extensive necroses on the surface and the cut-surface. Fig. 6 shows such a kidney in which histology revealed relatively intact glomeruli. In some kidneys there was some exudate staining with eosin. In most cases the tubular changes were severe, sometimes the lumen disappeared and round-cell infiltration was observed.

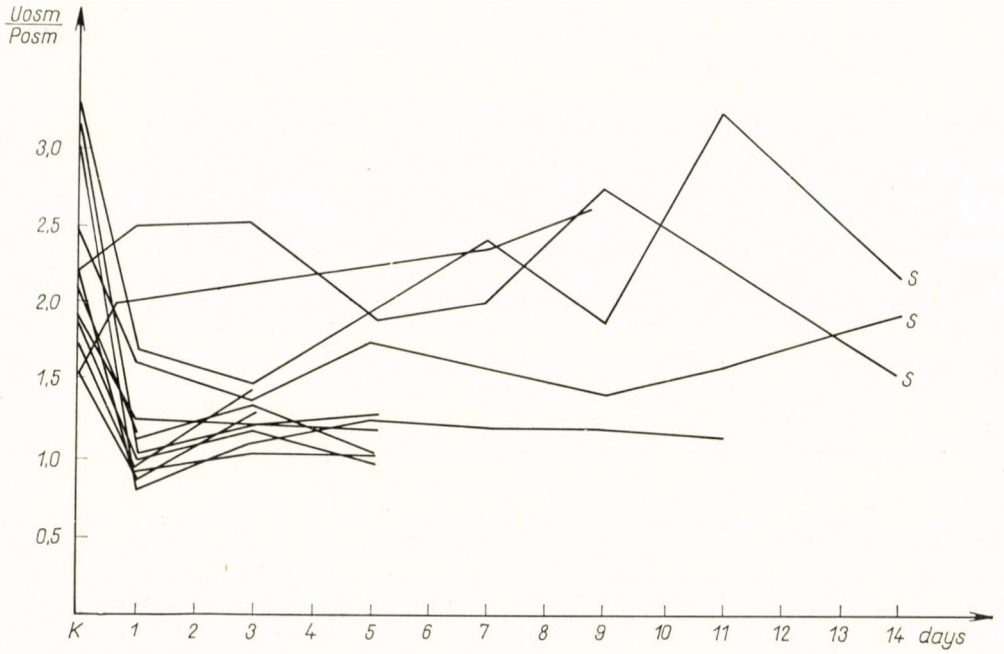

Fig. 4. Ratio of serum and urine osmolality. Signs as in Fig. 1

Discussion

The results have made it evident that experimental acute renal failure develops if the renal artery has been clamped under superficial anaesthesia or in the unanaesthetized animals. On the basis of these findings, morphine cannot be responsible for the renal damage.

In previous experiments animals subjected to deep anaesthesia survived the procedure but superficially anaesthetized animals did not. Considering that the latter animals showed signs of pain, we assume that the pain was one of the most important factors causing renal damage. In the present experiments ischaemia was brought about without causing pain, nor did the animals show

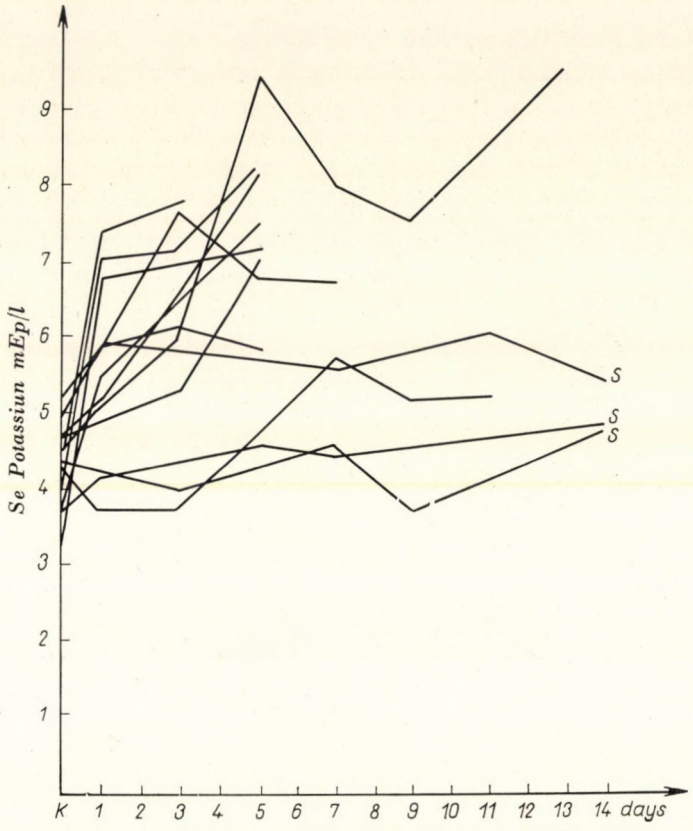

Fig. 5. Serum potassium level, mEq/litre. Signs as in Fig. 1

Fig. 6. Kidney after two hours arterial clamping without anaesthesia. Cortex shows signs of ischaemia; the medulla is dark and congested

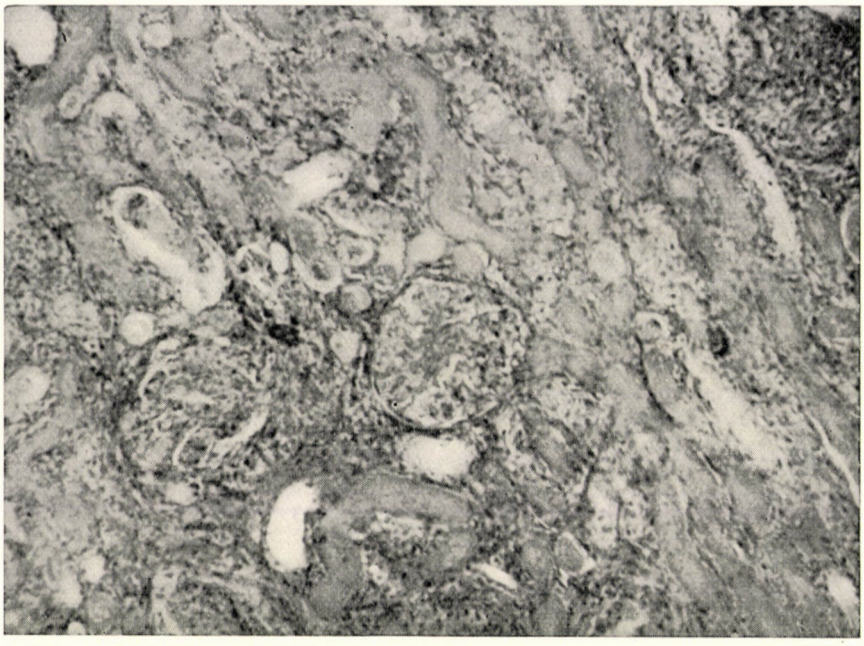

Fig. 7. Histology of the kidney in Fig. 6. Note comparatively unaffected glomerules and necrosed tubules

signs of pain during the two hours of renal artery clamping. The fact that renal failure developed in these animals, too, proves that pain alone does not cause renal damage in this experimental model.

On the basis of the present and previous experiments, it is assumed that a reflex starting from the ischaemic area, in this case from the kidney, causes some circulatory disturbance. The kidney might be the most sensitive organ, and then the reflex causes a disturbance in renal circulation. On inhibiting the reflex the damage does not develop. Such disconnection can be produced by deep anaesthesia or by chlorpromazine treatment, as well as by cutting the splanchnic nerve. A similar mechanism may operate in the development of the crash syndrome where the reflex starting from a crushed limb brings about the circulatory disturbance in the kidney.

On the basis of the above findings it seems likely that human renal failure and the present experimental renal failure are similar conditions. Characteristically identical are the high non-protein nitrogen level, the severe uraemic condition and the increased serum potassium level, together with a decrease of urine flow and endogenous creatinine clearance. Also typical is the loss of tubular concentrating capacity, reflected in the production of isosmotic urine.

We had three animals which survived the injury. This finding may be explained by assuming the presence of a well-developed collateral circulation,

as has been shown to exist independently of the system of the renal artery (BÁLINT [2], SHEEHAN and DAVIS [27, 28]). The three survivors might have had such a well-developed collateral system, ensuring the renal blood supply in spite of the renal artery being clamped. In this way, the ischaemizing reflex failed to occur.

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EFFECT OF ISCHAEMIA ON ADRENOCORTICAL CORTICOSTERONE SECRETION IN THE RAT

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By arresting adrenal blood flow for predetermined periods the influence of local ischaemia on the structure, weight and blood flow of the adrenals, furthermore on corticosterone secretion *in vivo* has been studied in the rat.

Clamping of the adrenal vessels for two hours resulted in extensive adrenal necrosis associated with cessation of corticosterone production. Clamping for one hour was followed by focal infarctions in the adrenal cortex and a reduction of corticosterone secretion. ACTH failed to elicit a secretory response; this indicates that ischaemia affects the responsiveness of the cortical cells to the stimulatory effect of ACTH. Clamping for half an hour, though causing no necrosis, was nevertheless sufficient for reducing corticosterone production.

It seems reasonable to assume that under pathological conditions, adrenal O_2 supply may influence the production of corticosterone.

The question whether corticoid secretion is affected by a reduction of adrenal oxygen supply is of theoretical and practical interest, and has been studied under various experimental conditions [1, 3, 5, 9, 12, 15, 17, 21]. Attempts have been made to induce systemic hypoxaemia by bleeding, experimental hypotension or shock, poisons interfering with cellular respiration, inhibition of pulmonary O_2 uptake, reduction of O_2 tension in the air. Laboratory animals in response to such factors exhibited enlargement of the adrenals with broadening of the inner cortical zones associated with an increased secretion of corticoids. Studies of adrenocortical activity carried out in human subjects in hypoxic conditions have led to similar results [10, 13, 18].

These investigations though having conclusively shown that systemic hypoxia was associated with increased adrenocortical activity, have failed to throw light on the question whether isolated reduction of O_2 tension in the adrenal cortex had any effect on corticoid secretion. It is highly debatable whether under the above discussed conditions it came to a cortical O_2 deficiency, and even if this was the case, it is partial, incalculable, unselective and therefore not standardizable. Obviously, generalized hypoxaemia sets various compensatory mechanisms into operation which may lead to a misinterpretation of adrenocortical function. We have only to remember in this respect the recent results reported by MARKS et al. [11] who have found that a reduction of O_2 tension in the air causes a significant increase in the ACTH content of the pituitary gland and the blood. This led them to attribute the adrenocor-

tical hyperactivity associated with hypoxia to an increased ACTH production rather than to a direct effect of hypoxia on the adrenal cortex.

In opposition to the foregoing investigations, we have attempted to ensure the following experimental conditions.

1. Unilateral adrenal hypoxia with unchanged O_2 supply to the whole organism;

2. Total anoxia of one of the adrenals;

3. Possibility of maintaining hypoxia as long as necessary.

This we expected to attain by temporary arrest of the blood flow to one of the adrenals, thus producing unilateral adrenal ischaemia. A simple device made it possible to withhold adrenal blood flow as long as necessary. Under the conditions thus produced, we studied the structural pattern and the weight of the adrenals, furthermore the corticosterone level in samples of adrenal venous blood.

Method

Female albino rats of identical breed, 200 to 250 g in body weight, kept on a standard diet were used. The animals were divided into eight groups. Those of the first group, serving as controls, were subjected to no operation. In preliminary experiments, some of the animals were subjected to a sham operation which, however, did not affect the results. Therefore no sham-operated group was included into the main experiment. In the animals of groups 2 to 7, the blood flow to one of the adrenals was occluded for half, one and two hours respectively by a procedure to be described below. The structure and weight of the adrenals as well as the corticosterone level in the blood of the adrenal vein were recorded one and 24 hours after release of the clamp. In the animals of group 8 the clamp was left in position for one hour and the studies were made 24 hours after its release. These animals received 2 IU ACTH (Exacthin-Richter) intravenously one hour before cannulation of the adrenal vein and the same amount by the same route when the cannula was inserted.

A clamp of the smallest size was adapted to the special purpose of inducing adrenal ischaemia, in a manner that its jaws consisted of two interlaced rings. Surgery was carried out under superficial ether anaesthesia, and the left adrenal gland was grasped in a manner to lie free between the two rings while the vein and the arteries of the periadrenal connective tissue were firmly compressed. Then the abdominal wall was closed by clips. At half, one, and 2 hours the instrument was removed through the laparotomy wound in superficial ether anaesthesia and the abdominal wall was again closed.

Clamping ensured full suspension of adrenal blood flow. On the evidence of our earlier studies [7] adrenal ischaemia induced in this manner is selective and complete. If, however, the clamp was left in position for one to two hours, after its release the adrenal vessels opened incompletely or not at all. There was therefore no way of ascertaining the actual duration of ischaemia, though it had been complete beyond any doubt.

The adrenals were examined one and 24 hours after release of the clamp. Previous cannulation of the isolated left adrenal vein had been performed in heparinized rats under pentobarbital anaesthesia and the venous blood flowing out through the cannula was collected during half an hour and measured with 0.1 ml accuracy. Corticosterone was estimated by the paper chromatographic method described by WEISZ and GLÁZ [20]. Details of the procedures used have been published earlier [2, 8]. Corticosterone values have been expressed in relation to the amount of blood collected from the adrenals (microg/ml), to adrenal weight (microg/hour/100 mg adrenal gland), and body weight (microg/hour/kg body weight). The first parameter defines the corticosterone concentration of adrenal venous blood and the two others, the total amount of corticosterone produced in a unity of time.

The left adrenal was removed after bleeding in each animal, carefully freed from adhering tissues, weighed on a torsion balance with 0.5 mg accuracy. The results were evaluated by Student's "t" test.

For histological study the adrenals fixed in 4 per cent formalin were embedded in paraffin, and sections 4 to 8 μ thick were stained with haematoxylin-eosin.

Results

Since the structural changes ensuing upon temporary adrenal ischaemia have been described earlier in full detail [7], the results of the present histologic studies will only be discussed in brief.

No significant changes were seen one hour after the clamp had been released. This was only to be expected, since no extensive necrosis could have developed during that time. In a number of animals focal oedema of moderate degree was seen in the cortical area. At sites the sinusoids were dilated and congested. Occasional loosening of columns was seen in the zona fasciculata. The most distinct changes appeared when clamping had been maintained for two hours; in this group adrenal oedema and congestion were fairly widespread, while in the cases where the clamp had been left in place for one hour, the changes were focal, variable in extent and degree, with initial signs of demarcated infarctions but no actual necrosis. After clamping for half an hour, adrenal structure did not differ from that in the controls.

The adrenals displayed characteristic changes 24 hours after release of clamping, particularly if the clamp had been left in place for two hours. In this latter case there was widespread cortical necrosis extending over the zona fasciculata and zona reticularis. In some of the animals the zona glomerulosa showed disintegration without any evidence of necrosis, while in others this zone too had completely disappeared. In some of the animals the medullar substance was necrotic, in others it remained alive. In the absence of medullar necrosis, the perimedullar cell layers of the zona reticularis preserved their structure. In some of the animals oedema of the medulla, cellular swelling and focal necroses were seen. Clamping for one hour also resulted in severe changes. While in these animals the zona glomerulosa and the medulla were free from necrosis, the zona fasciculata showed characteristic focal infarctions, with their wedges protruding into the zona reticularis and their bases in the outer zones of the zona fasciculata, but necrosis never involved the zona glomerulosa. Severity of the changes was variable. Some of the animals exhibited occasional minor infarctions confined to 5 to 10 per cent of the cortical area, while in others the foci were larger, had fused together and invaded 60 per cent of the cortex. If adrenal ischaemia had been maintained for half an hour only, no significant cortical changes and no necroses were seen 24 hours later. The only sequel of ischaemia in these cases was an occasional swelling of the cells of the fasciculate zone associated with a slight broadening of the cortex.

Adrenal weight, volume of adrenal venous blood and its corticosterone content are shown in Tables I and II.

Table I presents data obtained one hour after release of the clamp. One hour after clamping for half an hour the adrenal weight as well as the volume of adrenal venous blood did not change significantly, the corti-

Table I

Corticosterone production in vivo one hour after release of adrenal ischaemia

Group	No. of animals	Body weight g	Left adrenal mg/100 g body weight	Outflowing venous blood, ml/min.	Corticosterone		
					microg/ml	microg/hour/ 100 g adrenal weight	microg/hour/ kg body weight
I. Control	13	237.3 ±6.5*	13.0±0.6	0.15±0.01	2.7±0.2	77.2±2.6	97.1±6.5
II. Clamping ½ h.	12	233.0 ±6.2	13.3±0.5	0.12±0.01	1.4±0.2	33.0±5.4	44.4±7.6
III. Clamping 1 h.	13	237.3 ±5.3	14.6±0.5	0.09±0.01	1.4±0.2	25.4±4.8	33.6±5.8
IV. Clamping 2 hrs.	10	248.0 ± 5.3	18.7±1.1	0.05±0.01	0.2±0.1	1.9±1.1	3.6±2.3
*Standard error		I/II	p>0.05	0.05>p>0.02	p<0.001	p<0.001	p<0.001
		I/III	p>0.05	p<0.001	p<0.001	p≪0.001	p<0.001
Probability		I/IV	p<0.001	p≪0.001	p≪0.001	p≪0.001	p≪0.001

Table II

Corticosterone production in vivo 24 hours after release of adrenal ischaemia

Group	No. of animals	Body weight g	Left adrenal mg/100 g body weight	Outflowing venous blood ml/min.	Corticosterone		
					microg/ml	microg/hour/ 100 g adrenal weight	microg/hour/ kg body weight
I. Control	13	237.3 ± 6.5	13.0±0.6	0.15±0.01	2.7±0.2	77.2±2.6	97.1±6.5
II. Clamping ½ h.	13	235.0 ±6.4	18.3±0.8	0.12±0.01	2.6±0.3	46.2±7.5	79.5±10.9
III. Clamping 1 h.	10	237.0 ±8.3	17.7±1.5	0.15±0.02	2.1±0.4	34.7±6.5	55.0±8.2
IV. Clamping 2 hrs.	10	219.0 ±5.6	19.5±1.2	0.04±0.01	0.1±0.1	1.7±1.2	3.0±2.0
V. Clamping 1 h. + ACTH	12	231.3 ±9.5	19.8±1.0	0.09±0.01	2.0±0.2	27.2±4.5	46.0±7.3
*Standard error		I/II	p<0.001	p>0.05	p>0.05	p<0.01	p>0.05
		I/III	p<0.01	p>0.05	p>0.05	p<0.001	p<0.001
		I/IV	p<0.001	p<0.001	p≪0.001	p≪0.001	p≪0.001
Probability		III/V	p>0.05	0.05>p>0.02	p>0.05	p>0.05	p>0.05

costerone level in adrenal venous blood fell to half its original level. The total amount of corticosterone whether calculated on the basis of adrenal weight or of total body weight was also significantly reduced. Clamping maintained for half an hour and one hour respectively lead to essentially similar changes. Corticosterone concentrations were identical in both groups. The only possible difference was a somewhat greater reduction in blood volume after one hour clamping, and this might have accounted for the greater reduction in corticosterone concentration. The changes seen one hour after clamping for two hours were marked. The weight of the left adrenal was significantly increased, its blood flow was significantly reduced, in most animals to such a degree that corticosterone could not be estimated. Necroses were still absent in this stage.

Table II presents findings obtained 24 hours after release of the clamp. Clamping for half an hour was followed by an enlargement of the gland and a slight reduction of the volume of adrenal venous blood, but the corticosterone concentration showed no difference from the control values. The total amount of corticosterone calculated for adrenal weight was reduced, while in relation to total body weight it did not differ from the control values. Clamping for one hour produced essentially the same changes. The adrenals were moderately enlarged, while their blood flow remained in the control range. Corticosterone concentration was slightly but not significantly reduced, while reduction of its absolute amount was significant both in relation to adrenal weight and to total body weight. In those animals which had received ACTH, no significant change, particularly no increase in corticosterone secretion was found 24 hours after clamping for one hour. Twenty-four hours after clamping for two hours, the enlarged adrenals yielded scarcely any blood, containing corticosterone in traces or none at all.

Discussion

The results suggested that the adrenocortical tissue is sensitive to hypoxia, as reflected by the severe damage and reduced hormone level after temporary occlusion of adrenal blood flow. However, it never came to total adrenal necrosis and cessation of corticosterone production unless hypoxia had been maintained for two hours. The changes seen after clamping for half an hour or one hour were confined to certain areas of the gland while in the others live tissue producing corticosterone was preserved. The adrenal cortex thus seems to have a relative resistance to hypoxia.

Adrenocortical hypofunction induced in this manner is unrelated to the pituitary gland, in other words, it is not the consequence of a decreased ACTH production, since the reduced corticosterone secretion was unaffected by massive doses of ACTH administered after release of the clamp. This conclusively

shows that hypoxia affects directly the corticoid-producing cells which lose their responsiveness to the stimulatory effect of ACTH, though this latter may be present in large amounts in the blood.

The seeming contradiction between our results and the published evidence in favour of an increased activity of the pituitary adrenocortical system in hypoxia, springs from the circumstance that in the present study it was not systemic but local hypoxia whose functional effects were investigated under experimental conditions entirely different from those under which hyperactivity had been observed. Generalized hypoxia may be regarded as a major stress causing ACTH hypersecretion and secondary adrenocortical hyperfunction [11]. Our findings clearly indicate that the consequence of adrenal hypoxia is a reduction in hormone production rather than hyperactivity.

The question of the correlation between adrenal blood flow and hormone production has been investigated by various authors [4, 6, 14, 15, 19, 22]. There is evidence [4, 19] suggesting an influence of adrenal blood flow on the gland's activity. The present experiments did not provide conclusive evidence in respect to such kind of physiological regulation of adrenocortical function. It appears, however, that in addition to ACTH, adrenal O_2 supply may also influence corticosterone secretion, at least under pathological conditions.

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ACUTE RENAL FAILURE INDUCED BY URATE INFUSION IN THE RABBIT

AN EXPERIMENTAL STUDY OF THE PART PLAYED BY URATES IN THE INDUCTION OF SHOCK-KIDNEY

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It has been shown in rabbit experiments that rapid renal excretion of parenterally administered uric acid results in intrarenal accumulation of urates and their deposition in the tubular epithelium with secondary renal injury.

Tourniquet shock significantly increases sensitivity of the organism to the nephrotoxic effect of uric acid.

On the evidence of the present studies, the susceptibility of man to renal failure in association with shock may be connected with the urate metabolism characteristic of the human organism.

The pattern of acute renal failure generally designated "acute tubular nephrosis", "lower nephron nephrosis", "crush syndrome", etc. had been described by BYWATERS [7] and OLIVER [17]. Its clinical picture is marked by the appearance of anuria one to three days after the acute cardiovascular phase of shock has been overcome. If the patient survives the successive stages, he may recover, but the syndrome is usually fatal. Death may ensue at any stage in consequence of uraemia or electrolyte imbalance.

Some of the eliciting factors — such as CCl_4, benzene, sulphonamides, free haemoglobin, — are directly affecting the kidneys, but in most cases shock or injury is the cause of the renal failure. According to present knowledge, the most significant factor in the production of shock-kidney is the shifting of renal blood flow elicited by the shock effect [21] and the secondary anoxic damage to the kidney [14]. Even in the absence of shock, temporary arrest of renal blood flow in laboratory animals results in lesions which may be regarded as a model of acute renal failure [1]. Nevertheless, the study of renal failure subsequent to shock has the limitation that though shock may be induced with any desired degree of severity in practically every mammalian species, no acute renal failure similar to that seen in human subjects develops in the stage of reparation. Though recently BÁLINT et al. [2] were able to induce renal failure in unanaesthetized dogs under particular conditions, nevertheless it is obvious that human beings are more vulnerable to the renal effects of shock than are laboratory animals. This fact has drawn our attention to the possible connec-

tion of these differences with the purine metabolism characteristic of the human organism. On the faith of published evidence, except for the dalmatian dog, the human being represents the only mammalian species which has no uricase enzyme. This is why human purine metabolism ends with uric acid while this compound in the species provided with uricase is broken down into allantoin. It may be presumed that deposition of uric acid may also play a part in the development of renal failure. Our previous investigations revealed uric acid to reach excessive blood levels in acute metabolic disorders, for instance in toxi-infectious conditions of infancy. Hyperuricaemia has been shown by VAN SLYKE [21] to accompany traumatic shock.

A further support of our supposition was the finding of FOLIN et al. [11] according to which there is a delay in the conversion of uric acid into allantoin when administered intravenously in the form of Li-salt. Nevertheless, it disappears rapidly from the blood and accumulates selectively in the kidneys. Though the renal injury induced by the parenteral administration of Li-urate was known by these authors, they did not link up this finding with the renal effect of shock, as this pathologic entity had not been known at that time. Actually they described the renal lesion as being similar to nephritis, alluding to the renal changes associated with gout. Later, in connection with two clinical cases, DUNN et al. [8] considered the effect of urate a possible factor in the production of the crush-syndrome, a supposition shared by BYWATERS and DIBLE [7] but ignored by later investigators.

We expected to gain more information about the role of urates in the causation of postshock renal failure by reproducing the clinical pattern seen in human subjects by means of urate infusion in healthy animals or still more effectively in animals in a state of reversible shock. It was hoped to attain this by choosing proper doses and suitable experimental conditions. The present report deals with our pertaining observations.

Methods

Adult male rabbits kept on a mixed diet of oats and vegetables were used. A group of animals received 1 per a cent solution of urate in 0.4 per cent Li_2CO_3, the other group a 2 per cent solution of urate in 2.75 per cent triethanolamine. The healthy animals received 0.20 g/kg urate into the marginal auricular vein at 90 min. intervals on four occasions, i.e. a total of 0.80 g/kg urate. The controls were injected with the solvent (LiCl and triethanolamine, respectively), volume, route of administration and intervals being the same as in the test group.

The animals were anaesthetized with 0.8 g/kg urethane intraperitoneally. One hour later, a tourniquet was applied high on the right hind leg for two hours. 30 minutes after release, the animals were given intravenously 0.02 g/kg uric acid dissolved in ethanolamine, at 60 min. intervals on four occasions, the total dose being 0.08 g/kg. Urine was collected in a metabolic cage, body weight was checked regularly. After death, the animals were autopsied, the kidneys were weighed, fixed in alcohol and in formalin.

In blood samples obtained from the marginal auricular vein and in urine uric acid was estimated by the method of BLAUCH and KOCH [6]. Uric acid content of renal tissue was estimated according to FOLIN et al. [9]. In serum NPN was determined by the hypobromite method,

Na^+ and K^+ by flame photometry, standard bicarbonate by ASTRUP's method modified by us [5]. Osmolarity of urine was calculated from the Na^+ and K^+ contents, its titratable acidity, total nitrogen and ammonia values. The figures thus obtained agreed with the osmolarity values yielded by cryoscopy within a 10 per cent limit of error.

Results

Urate-treated normal animals

General response to the infusion. Poor tolerance to the infusion of 0.8 g/kg urate was unmistakable; adynamia and severe dyspnoea were conspicuous. As seen in Table I, a number of animals died in consequence of the toxic effects of the substance within the first 24 hours. The animals which survived this period,

Table I

Survival and renal failure after infusion of urate and solvent

	Died within 24 hrs.	Died of uraemia after 24 hrs.	Azotaemia 100 mg per 100 ml < reparation	Azotaemia 100 mg per 100 ml >	No azotaemia	Total
0.8 g/kg uric acid dissolved in Li_2CO_3	4	5	1	—	—	10
0.8 g/kg uric acid dissolved in triethanolamine	6	5	2	6	—	19
LiCl control	—	—	2	2	4	8
Triethanolamine control	—	—	—	—	12	12
Total	10	10	5	8	16	49

became transiently symptom-free and started to feed. In spite of this, some lost considerable weight, and they developed progressive azotaemia leading to death. In the survivors, the subsequent course was marked by a gradual decline of azotaemia until the insult was finally overcome. Infusion of lithium urate and of triethanolamine urate was similar in effect, though the lithium salt proved slightly more toxic. This was due to the slight nephrotoxicity of the lithium ion as confirmed by the occurrence of azotaemia in part of the animals treated with the lithium solvent, while the ethanolamine solvent proved entirely harmless.

Changes in renal morphology and blood chemistry. The kidneys revealed conspicuous changes both in the animals which had died spontaneously and in those which had been killed by intravenous injection of air in the successive

stages of reparation. Swelling of the kidneys was found in those animals too which had been killed by air embolism. In those animals which died in the first 24 hours, gross swelling was present, and the cut surface of the kidneys which yielded oedema fluid in abundance, was streaked with urate crystals. Along the cortico-medullar boundary, the urate deposits were arranged in double streaks surrounded by a haemorrhagic area, while in the medullar region they were

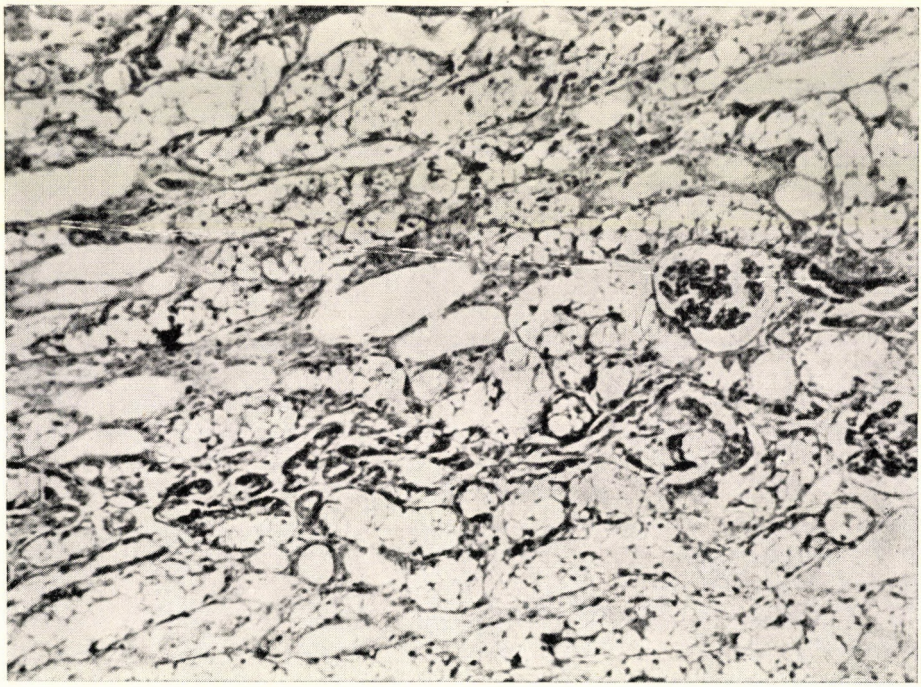

Fig. 1. Rabbit kidney 24 hours after infusion of 0.8 g/kg urate. Haematoxylin-eosin

arranged radially. The tubular epithelium showed grave changes with necrosis (Fig. 1). (The autopsy findings including histologic evidence will be dealt with in detail in another paper.) The described changes were, however, confined to those animals which had died or been killed within the first 48 hours. At later stages, the lesions were less severe and there were no urate deposits even in those animals which died of uraemia.

Fig. 2 presents renal weight in g/body weight in each group. Renal weight was highest in those animals which had died of uraemia between the first 24 and 48 hrs., but the kidneys were enlarged even in those animals which had overcome the azotaemic phase and were killed during the process of reparation. In the majority of the control animals, the kidneys weighed considerably less than in the urate-treated groups. The difference was still more marked if the

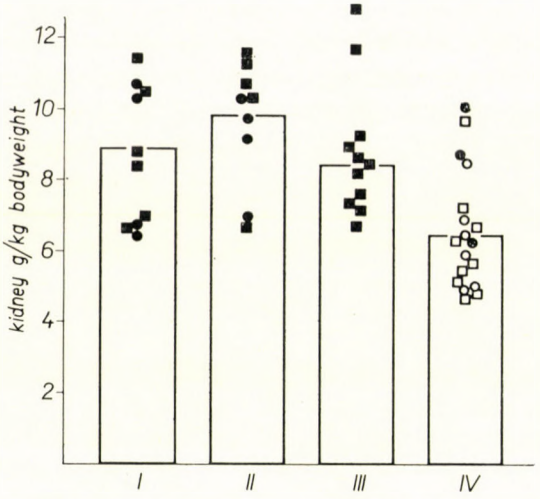

Fig. 2. Renal weight after urate and solvent infusion. ● = infusion of 0.8 g/kg Li-urate; ■ = infusion of 0.8 g/kg ethanolamine-urate; ○ = LiCl control group without elevation of NPN; ⊗ = LiCl control group, with elevation of NPN; □ = ethanolamine control group; I = died within 24 hours; II = died with uraemia after 24 hours; III = azotaemia followed by reparation; IV = controls

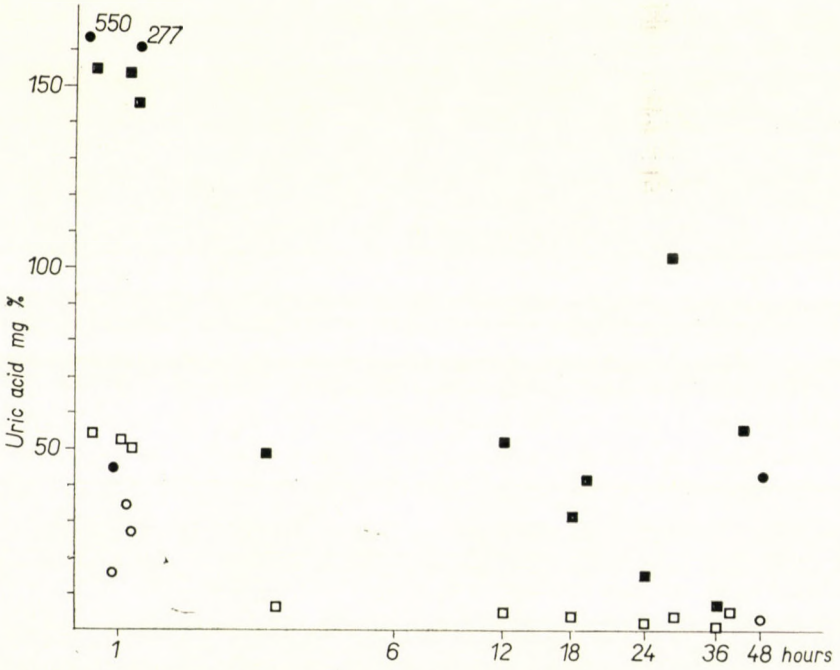

Fig. 3. Uric acid contents in kidneys and blood serum of rabbits after infusion of 0.8 g/kg urate. ● = Uric acid contents of the kidneys after infusion of Li-urate and ■ ethanolamine-urate; ○ = serum uric acid level after infusion of Li-urate and □ ethanolamine

abnormal figures occurring in the controls, obviously as a consequence of the effect of lithium, were disregarded.

Uric acid content of the kidneys (Fig. 3) of the animals which had died in the first hours was excessive; in the following hours it declined fast and after the first 48 hours the kidneys were practically free from uric acid (this latter finding has not been included into the Table). It further emerges from Fig. 3 that even in the first hours when renal uric acid content was at its peak, it was distinctly lower in the blood; two hours after the urate infusion, the blood uric acid levels were below 1 mg per 100 ml, the level typical of rabbits.

Blood chemistry and urine output. Table II shows the results in the same order as Table I. It may be seen that uraemic death ensuing within the first 24 hours was associated with a considerable rise in the serum NPN level, even in the cases of early death. Even in these cases, the increase in the urate N level was insufficient to account for the high NPN values. The increased NPN levels were accompanied with severe hyponatraemia, hyperpotassaemia and acidosis. These changes were more pronounced in the animals treated with triethanolamine urate.

Azotaemia was still more marked in the animals which had survived the first 24 hours but eventually died of uraemia. Here, too, hyponatraemia, hyperpotassaemia and acidosis were pronounced, but hypopotassaemia was also encountered. In this group observation of diuresis was possible. There was no phase of anuria, and urinary output neither increased nor decreased after the infusion, but the initial osmolarity of urine mostly declined to definite isosthenuria. The changes associated with the use of triethanolamine urate and of Li-urate were similar. In all but one of the cases attaining the stage of reparation, ethanolurate had been used. The highest NPN values were found in the samples taken on the fourth and sixth days, respectively, the highest value was 192 mg per 100 ml.

In Table II the data on blood chemistry and urinary output of the controls treated with triethanolamine are also seen. The only deviation from normal was the high standard bicarbonate on the second day; this was obviously due to the alkalizing capacity of ethanolamine as an organic amine. The other data tended to remain inside the normal range. In the animals treated with LiCl alone, azotaemia developed more gradually than after urate administration and these animals subsequently recovered.

Administration of urate to animals with mild shock produced by tourniquet of a limb

General response. Strangulation of one extremity for two hours usually does not elicit shock, and the intervention was tolerated without any manifest ill-effect. A 0.08 g/kg dose of uric acid, in other words, one tenth of that

Table II

Blood chemistry and urinary output of rabbits after infusion of urate and solvent

			No. of animals	Serum				Urine	
				NPN mg per 100 ml	Na+	K+	Stand. bic.	Volume ml/day	m Osm/l
					meq/l				
				Limit and average values					
Terminal values in animals which died within first 24 hrs.		After Li-urate	4	78.0—90.5 88.7	111—137 122	—	8.5—19.3 15.3	—	—
		After triethanol urate	6	81.0—162.6 124.0	109—145 130	4.8—10.0 7.4	8.0—18.8 14.2	—	—
Died of uraemia after 24 hrs.	After Li--urate	2nd day	5	64—222 136	119—138 127	3.3—10.0 5.8	13.3—19.0 15.9	110—135 118	350—718 440
		4th day	4	84—231 173	119—136 129	2.6—8.2 5.3	11.4—17.3 14.4	55—270 175	376—816 532
		6th day	3	105—352 237	128—140 136	— —	8.2—10.2 9.2	108—210 159	—
		8th day	1	295	121	8.2	8.2	—	—
	After trieth. urate	2nd day	5	83—185 132	127—141 134	4.6—10.0 7.9	10.9—22.0 15.9	30—237 162	—
		4th day	1	76	143	3.7	—	130	778
Azotaemia followed by reparation		2nd day	9	48—115 73	125—151 140	3.9—5.2 4.5	18.2—23.5 21.7	85—245 135	570—1020 695
		4th day	9	42—192 88.5	132—153 143	4.1—26.1 4.6	17.4—26.1 22.6	85—300 155	600—1100 770
		6th day	9	39.0—121 67.1	130—156 142	3.4—4.6 3.9	12.2—22.9 20.8	75—420 215	—
Infusion of triethanol-amine, controls		2nd day	12	24.0—35.4 29.6	139—147 142	3.2—4.2 3.9	21.1—29.7 24.7	70—500 210	—
		4th day	12	30.9—45.3 36.0	139—145 143	3.6—4.5 4.0	14.4—22.8 18.8	95—450 220	—
		6th day	12	25.8—44.7 34.0	130—144 142	3.2—4.9 3.9	17.9—22.4 20.6	70—580 220	—

applied in the previous group, was also smoothly tolerated. However, the same dose when administered after release of the tourniquet elicited a severe general response with extreme dyspnoea leading to death within a few hours before recovery from urethane anaesthesia. The animals surviving the first period were prostrated during two days and fed poorly even later. Azotaemia of variable degree ensued in all of the cases, but, as Table III shows, in the animals surviving the first 24 hours, it soon normalized.

Table III

Survival and renal failure after tourniquet-shock and infusion of urate and solvent

	Died within 24 hrs.	Reparation from 100 mg per 100 ml< azotaemia	100 mg per 100 ml> azotaemia	No. azotaemia	Total
Tourniquet on two hind legs for 90 min. + 0.1 g/kg ethanolamine urate	6	—	—	—	6
Tourniquet on a single hind leg for 2 hrs. + 0.08 g/kg ethanolamine urate	5	4	9	—	18
Tourniquet on a single hind leg for 2 hrs. + ethanolamine solvent, controls	—	—	—	3	3
0.08 g/kg ethanolamine urate without tourniquet	—	—	—	6	6
Total	11	4	9	9	33

Renal morphology and blood chemistry. The kidneys displayed changes similar to those seen after the administration of major doses of urate, particularly as regards the streaked pattern of urate deposits on the cut surface. The changes were confined in this series, too, to the cases with the shortest survival, but, in opposition to the other groups, no swelling of the kidneys was seen. A slight enlargement of the kidneys persisted after normalization of the azotaemic condition, while in the control animals which had been subjected to treatment with small doses but to no extremital strangulation, enlargement of the kidneys was considerable (Fig. 4). Fig. 5 shows the renal uric acid contents after infusion of 0.08 g/kg uric acid following release of tourniquet. Uric acid contents of the kidneys of these animals was higher despite the smaller dose of urate than in those presented in Fig. 3 which, though having received a massive urate dose, were not in shock.

NPN-level and urinary output. In this group, the peripheral circulatory failure subsequent to the extremital ischaemia made adequate sampling impos-

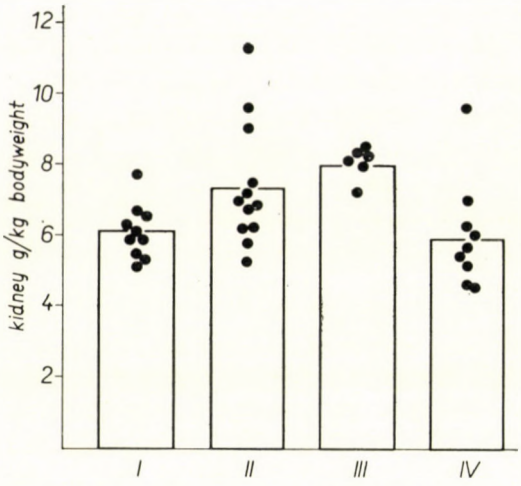

Fig. 4. Renal weight in rabbits after tourniquet on a single limb for 2 hours and perfusion with ethanolamine urate. I = Died within 24 hours; II = azotaemia followed by reparation; III = infusion of 0.08 g/kg ethanolamine-urate, no tourniquet; IV = infusion of ethanolamine after tourniquet

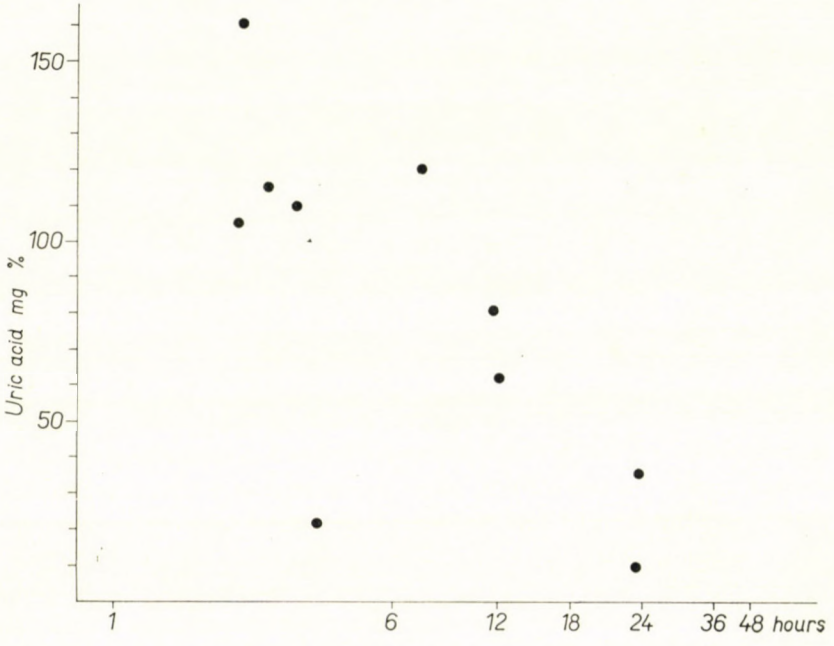

Fig. 5. Uric acid content of the kidneys in rabbits after tourniquet and infusion of 0.08 g/kg urate

sible and restricted us to studies of the NPN-level. Table IV shows that in the animals which died within the first 24 hours, azotaemia was distinct by the end of two hours. The responsible factor was renal injury caused by uric acid, since the tourniquet shock was never followed by any significant elevation of NPN at this stage unless uric acid had also been administered.

Table IV

NPN and urinary output after tourniquet shock, and urate and solvent infusion

	No. of animals	NPN, mg per 100 ml			Urinary output ml/day		
		2nd day	4th day	6th day	2nd day	4th day	6th day
Died within 24 hrs.	11	65—91* 79	—	—	anuria	—	—
Azotaemia >100 mg per 100 ml followed by reparation	4	92—131 106	65—146 99	61—64 63	anuria	70—150 120	60— 80 87
Azotaemia <100 mg per 100 ml followed by reparation	9	45.3—66.9 54	43.4—89.0 61	42.5—75.6 51	40—235 125	55—270 100	130—260 190
Tourniquet on a single limb for 2 hrs without urate infusion	3	34.0—36.0 35	22.8—30.6 27	26.4—31.8 29	75—150 110	50—380 250	140—425 290
Infusion of 0.08 g/kg ethanolamine urate without tourniquet	6	39.6—57.0 48	33.0—50.4 42	29.4—36.5 32	50—180 110	125—200 150	70—170 140

* Sampling 2 hrs. after administration of urate.

In 4 out of 24 animals submitted to extremital ischaemia and to urate perfusion NPN rose above 100 mg per 100 ml and persisted at these high levels until the sixth day when it started to decline. The animals were anuric during the first 48 hours and continued to have a restricted urinary output even later, perhaps owing to a low fluid intake. The other 20 animals of this group also developed azotaemia though of a slighter degree, while their urinary output remained within the values of the controls.

As to the controls (Table IV), the only finding to be mentioned was a slight elevation of NPN when urate had been given in small doses without extremital strangulation.

Discussion

On the evidence of our findings a high rate of urate excretion results in intrarenal deposition of urates and to secondary renal injury. Transitory extremital ischaemia greatly increases the vulnerability of the kidneys by urate. The nephrotoxic influence of urates has been pointed out by FOLIN et al. [11], later by VONDRA et al. [23]. We have contributed to their findings by the evidence of progressive renal failure appearing in the course of the first days after urate infusion, furthermore we have shown that extremital ischaemia increases the sensitivity to the nephrotoxic effect of urates, pointing out the connection of these phenomena with the renal failure associated with shock. Our claim that the urates play a part in that condition has been supported by the progressive renal failure elicited by urate. Renal injury due to endogenous hyperuricaemia during shock may thus be assumed to be one of the factors of the renal failure. Liability of the human kidney to deposition of urates in conformity with the results of the present animal experiments has been observed by FOLIN. In view of the high uricase activity of animal organs including the kidney, we had to use massive doses of urate in order to cause renal lesion. However, the amounts of endogenously released uric acid in the human subject are close to the quantities causing renal damage after extremital ischaemia. A further point in case is the common association of gout with renal failure [13] or the high incidence of acute renal failure in leukaemia or in metabolic disorders, as a consequence of massive uric acid release and of its deposition in the kidneys [15, 19]. The factor in our experiments responsible for the increased vulnerability of the kidneys was in all probability the extremital ischaemia, in connection with the consequential circulatory changes. The shifting of renal blood flow invariably demonstrable in experimental shock is insufficient to cause renal failure, unless there is an additional accumulation of urates to which the human organism is liable. Accordingly, the accumulation of uric acid seems to be essential in the development of renal failure consequential upon shock in man, but by no means its only cause. All of the factors conclusively proved to be involved in the shock mechanism — anoxia, disturbances of blood flow, nervous, ADH [26] mechanisms, etc. — may equally share in its production by promoting the damaging effect of uric acid on the kidneys. Acidosis and acidity of urine are, presumably, further contributive factors of importance. It must be remembered that ethanolamine which was used as a solvent in the present studies, is strongly alkaline and has a high buffer potency, therefore it is supposed to convey a certain protection to the kidneys from the toxic effects of uric acid rather than to cause harm.

The question arises why no post-mortem evidence of urate deposition has thus far been reported in cases of post-shock renal failure. According to our observations to be reported in a further paper, there actually is a substantial

deposition of urates in these cases, but for their demonstration fixation in alcohol and special staining are necessary, since the urates are dissolved during the usual procedures. According to the present results, no significant intrarenal urate deposits can be detected when the first days after shock have passed.

When interpreting our findings we may well ask whether the renal injury caused by uric acid was of a toxic character, similar to the effect of other renal poisons. This is doubtlessly so, and it may be added that the other renal poisons — sublimate, uranyl acetate, sulphonamides, oxalic acid — are assumed to act in the same manner as does uric acid, namely through their selective extraction, deposition and excretion by the kidneys. The significance of uric acid in the present context lies, however, in its being a product — and in man even the end product — of normal metabolism.

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STUDIES ON ANTIDIURETIC HORMONE

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JEFFER's bioassay for ADH estimation in blood has been adapted to permit recovery of 1 micro-unit in 1 ml of plasma.

In normal well hydrated people, no antidiuretic activity is found in the blood. The functional capacity of the neurohypophysis can be tested by the intravenous injection of 20—25 ml of 10 per cent NaCl solution. The plasma ADH level then rises to 15—30 μu/ml in 20 min. A similar response can be elicited by the inhalation of amyl nitrite. Amphetamine and atropine inhibit the release of ADH even when osmoreceptors have been stimulated through increased osmolarity. Amphetamine may therefore be used in the treatment of primary hyperadiuretism, and sometimes also in premenstrual tension.

Enzymatic inactivation of ADH plays a role in some cases of secondary hyperadiuretism. In men and non-pregnant women no vasopressinase activity can be demonstrated in the blood. In pregnancy this activity begins to appear at the end of the 2nd month. The peak is reached at 7 months, with a sharp fall after delivery. The enzyme is of placental origin. In toxaemia of pregnancy, however, its output is deficient. As a consequence, the blood ADH level begins to rise.

Liver vasopressinase is inhibited in severe hepatic insufficiency. Some rise of the blood ADH level may occur in untreated myxoedema, due to deficient ADH inactivation in peripheral tissues.

The neurohypophyseal hormones belong to the earliest types of internal secretion [5]. From the evolutional point of view they are successors of primitive neurohormones present already in insects. This paper will deal solely with vasopressin (adiuretin, antidiuretic hormone, ADH). Its existence in the earliest stages of phylogenesis emerges from the fact that contrary to other hypothalamic hormones its target organ is not the pituitary but peripheral tissue, i.e. the renal tubuli. It was ADH which during the evolution enabled the living matter to leave water and survive on dry land.

In spite of its simple structure ADH is a potent agent. As little as 1 mg of the pure substance of arginine-vasopressin contains 500 I. U. of ADH. Since in normal human subjects 0.2 μg i.e. 0.1 miliunit is capable of eliciting a full antidiuretic effect lasting for about 45 minutes, 1 mg of ADH would be sufficient for the induction of antidiuresis in 5000 individuals. It follows that one I. U. of ADH equals 2.0 μg. In a hormone with this degree of potency it should be anticipated that the organism effectively controls both its release and its inactivation.

The release of ADH into the circulation is controlled by the vegetative nervous system which in turn is stimulated by osmoreceptors (and to a lesser

degree by volume-receptors). Inactivation of ADH is enzymatic, main sources of the enzymes being liver, placenta and kidneys.

Our studies have dealt both with the release and with the inactivation of ADH.

Methods

So far no biochemical methods have been developed which would provide the possibility of estimating minimal concentrations of ADH in body fluids. Several methods of bioassay were devised, the sensitivity of which was, however, unsatisfactory. They allowed the determination of ADH plasma levels only at titres exceeding 30 μu per 1 ml. However, in normally hydrated individuals 1 ml of plasma contains only exceptionally more than 1 μu of ADH. Another disadvantage of the earlier methods was the large amount of blood required for titration. For clinical purposes we needed a method which would require minimal amounts of blood and which would be more sensitive than earlier methods. We have therefore modified the method described by JEFFERS [13] who measured antidiuresis in rats anaesthetized with 5 ml of 12 per cent alcohol after administration of 3 ml of water. This required surgical intubation of the urinary bladder. The method allowed to estimate concentrations from 20 up to 30 μu ADH in 1 ml of plasma. In our modification male rats are used which are fasted but have free access to water. The animals must previously be adapted to their environment for at least three weeks. For the induction of anaesthesia we use exact doses of alcohol: two doses of 3 ml per 100 g body weight of 12 per cent ethanol. Continuous rehydration is carried out throughout the experiment by the repeated administration of water in doses corresponding to 1 per cent of body weight according to the amount of diuresis. In this way it is possible to carry out as much as 10 estimations in the same rat. We plot on a chart the data of both the reduction of diuresis and the duration of antidiuresis, and by multiplying these values we obtain an area. This is then expressed in terms of per cent of another area the extent of which is determined by the onset of antidiuresis multiplied by a theoretical time of 60 minutes. We have selected this time period because even after the highest dose of ADH (500 μu) the ensuing antidiuresis never exceeded 60 minutes. This theoretical area serves, therefore, as a basis for the calculation of the actual antidiuresis which always lasts for less than 60 minutes. By this calculation we eliminate the error arising from the dependence of the antidiuretic response upon the degree of the artificially induced diuresis. The value of antidiuresis is compared with the effect of Voegtlin's standard powder.

This modification increased the sensitivity of the method up to 10 μu per ml of plasma. In an attempt further to increase the sensitivity, denervation of the kidneys was performed. The procedure was, however, rather complicated and not suitable for routine clinical use [18]. HELLER subsequently succeeded in improving the method by eliminating the surgical stress [7] in that instead of suturing the tube into the urinary bladder he prepared a permanent urinary fistula. In this way the mobilization of endogenous ADH was eliminated and sensitivity of the method increased up to 1 μu of ADH in 1 ml of plasma. This has been found satisfactory and we use HELLER's modification as a standard procedure.

Observations

In normally hydrated healthy individuals either no or at most 1—3 μu per 1 ml antidiuretic activity was demonstrated in the plasma. Both thirsting and/or ingestion of salt were followed by an immediate increase of the ADH level, provided that the neurohypophysis was capable of responding to increased osmolarity. Therefore, to begin with, an attempt was made to devise a test of the functional capacity of the neurohypophysis, to serve for the differentiation of true diabetes insipidus from primary polydipsia. For clinical purposes the most convenient stimulation of osmoreceptors is done through increas-

ing the osmolarity of the blood by at least 2 per cent. Samples of blood are taken from the cubital vein and immediately placed on ice. They must be used within 20 minutes. The rat, therefore, should be prepared in advance for titration. With the same needle 20—25 ml of a hypertonic (10 per cent) sodium chloride solution is injected into the vein. A second blood sample is taken after 20 minutes. Both samples are titrated in the same rat. Since for this as little as 0.7 ml sufficed, the blood loss is negligible and there is no major discomfort.

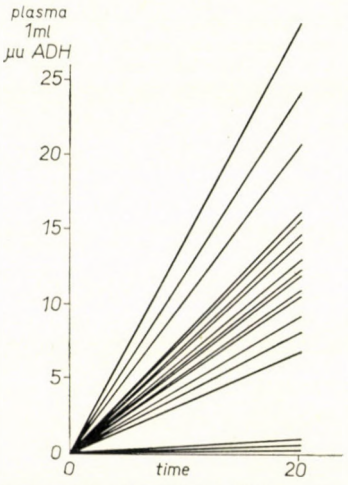

Fig. 1. 20—25 ml 10% NaCl intravenously

Fig. 1 illustrates several observations. Neither of the subjects showed a demonstrable plasma ADH activity prior to the injection of hypertonic saline, while 20 minutes later all of them displayed some increase, which often reached 25—30 μu per ml.

Clinical practice has shown, however, that this test is useful in diabetes insipidus in a negative way only. This is due to the fact that some primary polydipsias persisting for a long time are secondarily "insipidated". The continuous flooding of the organism by an excessive intake of water results in such an inhibition of ADH secretion that the neurohypophysis loses its capacity to react quickly upon an increase of osmolarity in blood. Thus, if in a case of polyuria of unknown origin the injection of hypertonic saline fails to increase the blood ADH level, the condition is either diabetes insipidus or a primary polydipsia of long duration. When, however, the injection is followed by a rise of the ADH titre, diabetes insipidus can be safely excluded.

Influence of the nervous system

So far no agreement has been reached whether the nervous system was capable of influencing the production and/or the release of ADH by acting directly upon the supraoptic nuclei, or whether it acts indirectly by the way of e.g. circulatory shifts which in turn would stimulate the volume-receptors or osmoreceptors.

Circadian rhythm. In contrast to numerous other hormones we have failed in providing evidence of a rhythmical release of ADH with distinct differences between the diurnal and nocturnal values.

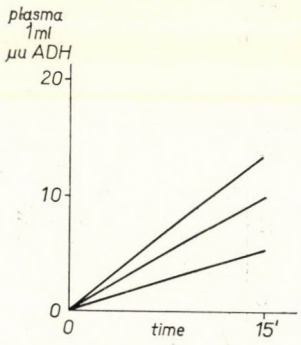

Fig. 2. Influence of induced anxiety

Conditioned reflex. We demonstrated its presence in human subjects [1, 2]. Its degree was, however, moderate. The experiment provided evidence of the nervous system being capable of directly influencing the production and release of ADH without the mediation of osmoreceptors or volume-receptors. The practical significance of this finding remains to be clarified.

Alarm reaction. A mental stress alone is sufficient for ADH mobilization. Fig. 2 illustrates the effect of an embarrassing interview on the blood ADH level, in three subjects. A feeling of anxiety prior to injection and pain during the injection may result in a similar ADH mobilization. Thus, an alarm reaction could well distort the result. Therefore we determined the blood ADH level at random in 100 normally hydrated individuals. Out of these in 19 emotion or pain during the injection resulted in a rise of ADH above 1 μu/1 ml. The highest titre was 8 μu/ml. In the remaining 81 subjects, however, no ADH activity was demonstrated. Thus in the majority of cases the emotion and the slight pain associated with the collection of blood samples did not interfere with the humoral mechanism of ADH mobilization. In spite of this it is necessary to take into consideration the subjects who do react. In clinical practice, therefore, only titres exceeding 10 μu/ml are classified as pathologically increased. In addition, only patients should be tested for ADH who are already adapted to

hospital environment and injections, and in whom the occurrence of an alarm reaction is therefore less probable.

Influence of drugs. Many drugs are capable of stimulating or inhibiting ADH production and release. The stimulants include nicotine, acetylcholine, morphine, phenobarbital, ether, ATP, ferritin, hexamethonium, etc. We suc-

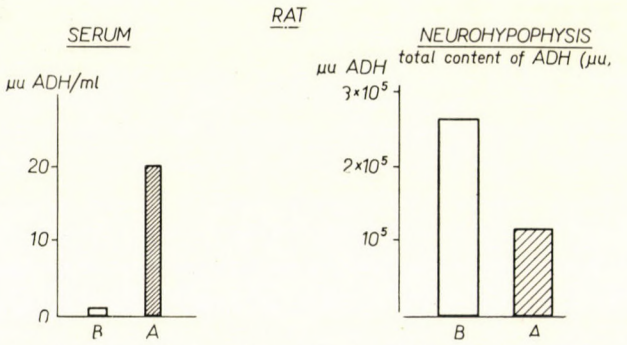

Fig. 3. ADH content before (B) and after (A) inhalation of amylnitrite

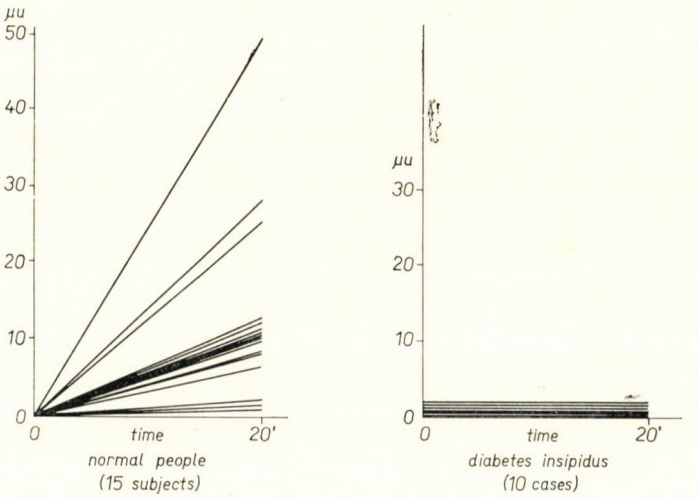

Fig. 4. Mobilisation of ADH after amylnitrite

ceeded in demonstrating that amylnitrite also acts as a stimulant [10, 11]. Fig. 3 shows the influence of amylnitrite inhalation in rats. The ADH store in the neurohypophysis decreased by more than fifty per cent, while the blood level increased. The release of ADH from the neurohypophysis into the circulation was therefore increased. In human subjects the inhalation of 0.03 g of amylnitrite may be tried as an auxiliary method of diagnosing diabetes insipi-

dus. Fig. 4 illustrates the response of 15 normal individuals as compared to 10 patients suffering from diabetes insipidus in whom no increase of the ADH level occurred. The mechanism of this action remains obscure. It is not induced by the decrease in blood pressure. We are not even certain whether vasodilatation is involved as another vasodilating agent, nitroglycerin, fails to influence ADH.

The best known inhibitors of ADH release are water and ethanol. We have shown that amphetamine, atropine and reserpine have a similar influence.

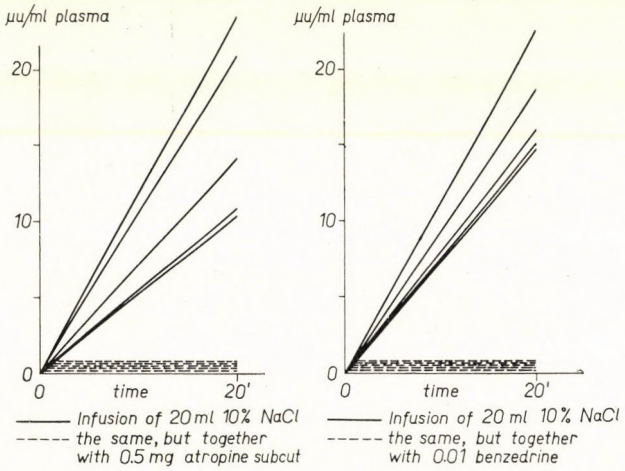

Fig. 5

Amphetamine and atropine were tested in normal subjects who served as their own controls [3, 4, 5]. We performed at first the routine test with hypertonic saline and repeated it three days later after the subcutaneous injection of 0.5 mg atropine or 0.01 g amphetamine. In spite of the increase of osmolarity after the hypertonic saline, no increase in ADH level was observed (Fig. 5). In other words, pharmacological stimulation of the sympathetic or inhibition of the vagus blocks the mobilization of ADH. This finding has a practical application. We use amphetamine wherever a high ADH level participates in the clinical symptomatology. Atropine is less convenient because of its unpleasant side effects.

Reserpine was tested in rats. Fig. 6 shows that its administration caused a rise of ADH stores in the neurohypophysis, while the blood ADH level decreased. Thus reserpine by blocking the release of ADH into the circulation, has an effect opposite to that of amyl nitrite. So far we do not know whether the same holds true for human subjects and whether the finding is of clinical importance.

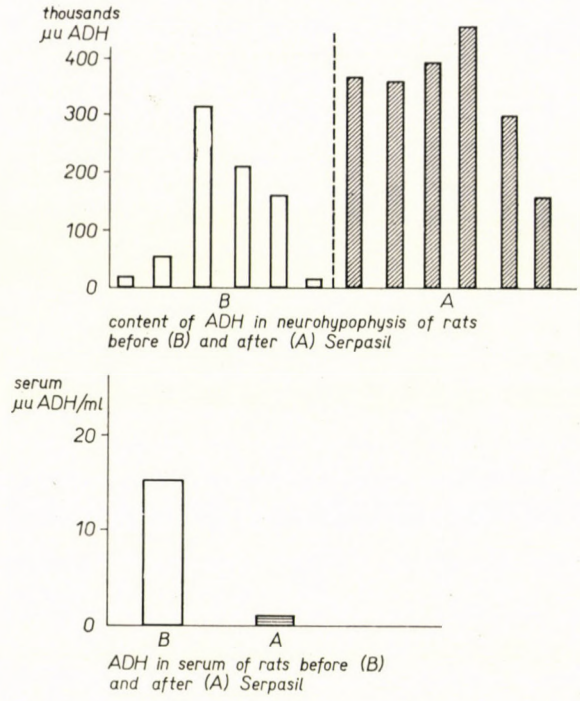

Fig. 6

Inactivation of adiuretin

ADH is inactivated mainly in the liver and the placenta, to a lesser degree in the kidneys and much less in other tissues. Under normal conditions it is not possible to demonstrate vasopressinase activity in plasma. We added to serum (or plasma) an amount of ADH to a concentration of 500 μu per ml. After incubation at 37 °C for 20 minutes, ADH was titrated back. The decrease of ADH activity was expressed in per cents of its original value.

In 10 normal men and 20 non-pregnant women the decrease of activity was either minimal, or in most cases absent. In 30 healthy pregnant women, however, the decrease of ADH activity was marked [12, 14, 15, 16, 17]. Some of the problems of ADH inactivation will be discussed below.

Clinical syndromes

Pathological conditions in which the clinical pattern is influenced by ADH may be grouped as follows.

(i) ADH deficiency: diabetes insipidus.

(ii) Target organ fails to respond: hereditary renal diabetes insipidus, potassium depletion, advanced pyelonephritis.

(iii) Excess of ADH: primary and secondary hyperadiuretism.

We cannot add new knowledge to the clinical syndromes (i) and (ii) except perhaps the above-mentioned functional tests with hypertonic saline or amylnitrite inhalation.

Hyperadiuretism

The existence of a primary hyperadiuretism was repeatedly suggested and again denied. Earlier clinical reports suffered from a basic shortcoming, i.e. the indirect estimation of ADH excess since no reliable method for its estimation was available. A theoretical objection against the concept of hyperdiuretism was the belief that a permanent excess of ADH would soon lead to water intoxication. From experiments with simultaneous administration of ADH and water we know, however, that after several days a steady-state sets in, during which hypervolaemia is accompanied by hyponatraemia, the kidneys regain their concentrating power and no water intoxication occurs. A chronic hyperadiuretism is thus theoretically possible.

Primary hyperadiuretism. The medical literature includes so far reports on about 30 cases. We have seen 3 patients with a verified diagnosis, and another patient in whom the condition was suspected. Three of our patients were females (ages 26, 36 and 44), one was a male (age 52). In neither of the females a relation between the symptoms and menstruation was established. In one of them the syndrome developed subsequent to infectious mononucleosis, in the male patient it had been preceded by a febrile illness of unknown (possibly viral) origin. In the remaining two females we failed to ascertain the cause of the condition. The clinical course was similar in all patients; it was characterized by intermittent water retention with thirst and oliguria, increase of body weight (sometimes with ankle oedema). All patients suffered from headache, fatigue, adynamia, and often from insomnia. One female had occasional seizures. After days or weeks, spontaneous diuresis had been re-established and all symptoms disappeared. All 4 patients prior to admission to our department had been treated elsewhere without success for migraine, etc., with various diuretics, hormones, analgesics or vitamins.

During the critical period an increased blood ADH activity was demonstrated in three patients. In one female we failed to prove it though her course as well as the therapeutic response were identical with those observed in other patients. Neither of our patients showed any major cranial or cerebral changes, but in three of them the EEG was altered during the period of increased ADH output. In all patients the serum sodium level was decreased during the critical period. All responded promptly to amphetamine therapy. The increased blood ADH level fell to unmeasurable values, sodium returned to normal, diuresis normalized, headache, fatigue and all other symptoms disappeared.

In our opinion, therefore, primary hyperadiuretism is an actually existing but rare clinical entity which responds readily to amphetamine, provided that there is no such underlying cause as a tumour, etc.

Secondary hyperadiuretism. In our concept this syndrome is due to sodium retention. Ensuing hyperosmolarity leads then to increase of ADH production and consequently to water retention. It is not clear, however, how far and how much can this compensating mechanism add to the clinical symptoms.

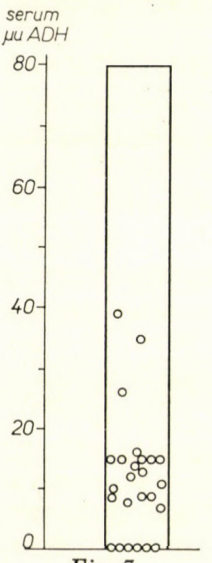

Fig. 7.
Premenstrual tension.
ADH level in the blood

We were interested in one of the syndromes included in this group, namely premenstrual tension. It is associated with retention of water resulting in an increase of body weight, with oliguria, anorexia or nausea, tension in the brests, sometimes psychic disorders, even suicidal attempts. The cause is sought in the ovaries. We observed 16 women. The curves of the basal temperature as well as the vaginal cytology were suggestive of frequent anovulatory cycles. Data concerning pregnandiol elimination were not available. These patients were followed up for several months. Plasma ADH level was estimated twice weekly. In five women no marked variation of the ADH titre was found, while in 11 women the titre showed a regular increase up to values of 37 μu per ml, 5 to 7 days prior to the onset of menstruation (see Fig. 7). During this time sodium retention was always present. Our observations are not yet concluded. Thus far, where routine treatment with saluretics and gestagens failed to act, a beneficial effect was observed after amphetamine and, surprisingly, also after oxytocin treatment.

Relative hyperadiuretism

In this interesting condition, ADH production is normal but its enzymatic inactivation is disturbed. Since vasopressinase is produced predominantly in the liver and the placenta, we studied the ADH levels in patients displaying disturbances of these two organs.

Liver. The study included 25 patients who had recovered from infectious hepatitis and in whom slight hepatic lesion persisted, and further 5 patients with advanced cirrhosis of the liver. The posthepatic subjects did not differ from the normal ones. The cirrhotics, however, presented some deviations. In all 30 patients diuresis was induced by water with simultaneous injection of 0.3 I. U. of ADH. Subsequently the delay of diuresis as well as the disappearance of exogenous ADH from blood were observed. The results were as follows.

	Posthepatic state	Advanced liver cirrhosis
Disappearance of ADH from blood	45′	60′
Delay of induced diuresis after ADH	60′	90′

The results were suggestive of a decreased hepatic vasopressinase activity in advanced hepatic cirrhosis, as shown by the delay in the inactivation of injected ADH as well as by the delay in the onset of induced diuresis. Vasopressinase deficiency was, however, not so considerable as to manifest itself in an increased blood ADH level; it was only apparent after water load, as a relative excess. This was not due to hyperosmolarity, the sodium levels in the patients were either decreased or normal. Advanced liver cirrhosis is, thus, not associated with hypersecretion of ADH but rather with a deficiency of its enzymatic inactivation.

Placenta. As already mentioned, we have investigated whether normal pregnancy was associated with an increase of the vasopressinase titre in blood. 50 samples of plasma (or serum) ADH were added to the concentration of 500 μu per ml. In an aliquot the added ADH was immediately titrated back. The second portion was incubated at 37 °C for 20 min. and the ADH titre was estimated subsequently. Decrease of activity was expressed in per cents of the value obtained prior to incubation. The course of this inactivation was studied throughout a normal pregnancy in 30 women. Plasma from 10 men and 20 nonpreignant women served as controls. Men and nonpregnant women were unable to nactivate ADH. On the other hand, in pregnant women the vasopressinase titre began to rise towards the end of the second month of pregnancy and reached its peak (up to 100 per cent) in the seventh month. After delivery it showed a sharp decline and disappeared completely within 4 weeks.

Thus, pregnancy is associated with a substantial increase of the ADH inactivating capacity of blood. In the seventh month 1 ml of serum is capable of inactivating as much as 500 μu of ADH in 20 minutes. Due to this ability it is not possible to demonstrate in these women ADH in the blood. The placental vasopressinase inactivates, however, only the free ADH. It does not influence the bound fraction which is capable of eliciting a response in the kidneys, too.

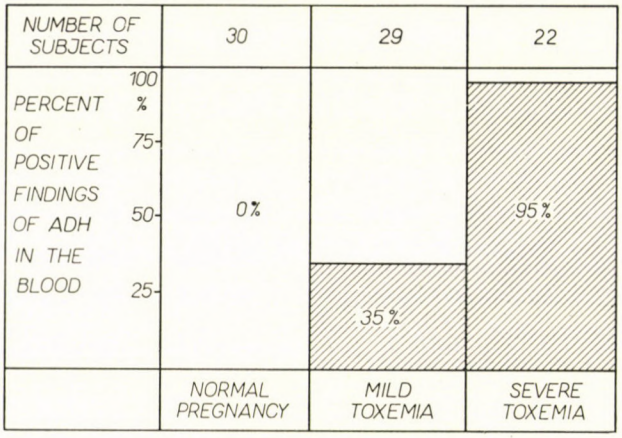

Fig. 8. Presence of ADH in the blood in pregnancy

Therefore no diabetes insipidus develops in pregnancy. The meaning of the increase of vasopressinase activity during pregnancy remains obscure.

Late toxaemia of pregnancy. The situation here is quite different, as is seen in Fig. 8. Altogether 51 patients were studied. Out of 29 women suffering from a mild form of toxaemia 10 showed an increase of the blood ADH level. Severe toxaemia was present in 22 women and out of these 21 had increased ADH levels. The increase was proportional to the severity of toxaemia. In preeclamptic toxaemia extreme values up to 100 μu per ml of plasma, or even more of ADH were found. This difference was so striking as compared to normal pregnancy that it has made us to suppose that ADH is in some way involved in the pathogenesis of toxaemia of pregnancy. The increase of the ADH level has a prognostic value; excessive titres indicate imminent eclampsia.

It is improbable that an overproduction of ADH should have taken place. The maintenance of an ADH level of e.g. 100 μu per ml of plasma would require approximately a thousandfold increase of its production by the neurohypophysis. This organ, however, does not possess such a high functional capacity. More probable is therefore a reduction of vasopressinase. And this again cannot be due to hepatic disorder. A certain deficiency of the enzyme is found in very se-

vere and advanced cases of hepatic cirrhosis only and hepatic lesions of this degree do not occur in toxaemia of pregnancy. This points to the role of the placenta.

We had therefore to ascertain whether the placenta itself cannot be the source of the excessive amount of ADH. Ten placentas from normal and 10 from toxaemic pregnancies were examined, but no trace of ADH activity was found in them.

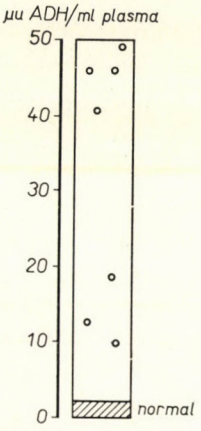

Fig. 9. ADH bood level in untreated myxoedema

Subsequently, blood vasopressinase activity was estimated in toxaemia of pregnancy in a similar way as described above. The difference was again striking. While the blood of women with normal pregnancy was capable of inactivating ADH very effectively, the blood of toxaemic patients of pregnancy has completely lost this capacity.

Thus we believe that placental vasopressinase is lacking in toxaemia of pregnancy which we therefore classify with the states of relative hyperadiuretism due to deficient enzymatic inactivation of ADH.

Thyroid. Hyperthyroidism is usually associated with higher diuresis, while in hypothyroid subjects urine output is less than in normal individuals. A possible connection with ADH seemed of interest. The estimation of ADH in the plasma of 7 patients suffering from myxoedema showed increased levels in all of them (Fig. 9). We had then to decide whether this was due to an increased production or to a decreased inactivation of ADH. Experiments were carried out in 12 male rats. Another group of 12 rats served as controls. The animals received methylthiouracil for 4 weeks. The artificial hypothyroidism gave rise by the well-known feed-back mechanism to an increased output of pituitary thyrotropic hormone. The question now was whether the

increase in TSH will be followed by a concomitant increase in ADH. The mean ADH content in the neurohypophysis was 158.000 μu in the controls and 180.000 μu in the rats treated with methylthiouracil. The difference did not reach statistical significance. ADH values in the blood were identical in the two groups.

The second experiment was carried out in human subjects. Ten individuals with a normal metabolic rate, 10 patients with thyrotoxicosis, and 7 patients with myxoedema received 20 ml of tea per kg of body weight, and subsequent diuresis was recorded. On the next day the experiment was repeated, yet this time with a simultaneous injection of 0.3 I. U. of ADH. This delayed the diuresis in normal individuals to 60 min., in thyrotoxicosis to 30 min., in myxoedema to 90 min. These findings are suggestive of the inactivation of ADH being accelerated in thyrotoxicosis and slowed down in myxoedema.

The next question to be decided was whether thyroxine acted directly on the kidneys, e.g. by increasing the sensitivity to ADH of the tubules. These studies were carried out in our laboratory by Pacovský [19]; they showed that thyroxine does not increase the sensitivity to ADH of the tubules. Our results thus suggest that thyroxine influences the inactivation of ADH in tissues. As compared to the placenta, this effect is of minor importance. It is, however, justified to include myxoedema into the category of relative hyperadiuretism.

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ТОМ XXIII — ВЫП. 1

РЕЗЮМЕ

ЧАСТИЧНАЯ ДИССОЦИАЦИЯ ПРАВОГО ЖЕЛУДОЧКА (МЕХАНИЧЕСКАЯ АТРИАЛИЗАЦИЯ) ПРИ АНОМАЛИИ ЭБШТЕЙНА

К. ЛОЖАДИ и К. ШАРКЁЗИ

Авторы наблюдали при произведении операции по поводу аномалии Эбштейна функциональную атриализацию анатомически атриализованного проксимального правого участка желудочка, причем упомянутый участов желудочка совершал с предверниями синхронные преддверные сокращения. Изменения ритма вызвало падение кровяного давления, которое удалось нормализовать лишь вливанием норадреналина.

Авторы стараются, на основании косвенных доказательств, сообщенных в специальной литературе, найти объяснения для указанных гемодинамических изменений, вызванных нарушением ритма.

ИЗМЕНЕНИЯ СОДЕРЖАНИЯ РИБОНУКЛЕИНОВОЙ КИСЛОТЫ В ПЕЧЕНИ КРЫС ПОД ВЛИЯНИЕМ ХЛОРИСТОГО АММОНИЯ

А. БАШ и Ф. ЙО

После введения 0,1 г хлористого аммония через день в течение 20 дней авторы исследовали у животных гистохимическим, биохимическим и электронномикроскопическим методом изменения печеночной ткани. Гистологическим методом исследования была выявлена повышенная пиронинофилия клеток печени. Под электронным микроскопом наблюдалась богатая организованная эргастоплазма, а количественным методом было отмечено повышение концентрации рибонуклеиновой кислоты на 24,8%, по сравнению с контрольными животными. На основании вышесказанного авторы предполагают, что введение хлористого аммония, в указанной выше дозе, вызывает в клетках печени повышение синтеза белков.

ДЕЙСТВИЕ ЭКСТЕРОЦЕПТИВНЫХ РАЗДРАЖЕНИЙ НА КРОВООБРАЩЕНИЕ НОРМАЛЬНЫХ КРЫС И КРЫС С ГИПЕРТОНИЕЙ ГРОЛЛМАННА

З. САБО, Л. ТАКАЧ, Т. ГАТИ и К. ДЬЕНГЕ

В кровообращении нормальных крыс и крыс с гипертонией Гроллманна (в бодрствующем или наркотизированном состоянии) наблюдаются следующие изменения минутного объема, распределения минутного объема и кровяного давления:

1. По сравнению с наркотизированными контрольными животными для кровообращения бодрствующих крыс характерно значительное повышение минутного объема, перераспределение минутного объема (высокая костно-мышечная фракция при низкой почечной и висцеральной фракциях).

2. Оценка изменений показателей кровообращения, наблюдаемых при гипертонии Гроллманна, затруднялись у бодрствующих животных стрессом от возбуждения, а у наркотизированных крыс значительным понижением гипертонического кровяного давления. Эксперименты авторов указывают на то, что в развитии гипертонии Гроллманна может участвовать также высокий минутный объем, в то время как повышенная коронарная фракция бодрствующих животных с гипертонией и повышенная костно-мышечная фракция наркотизированных крыс гипертонической группы указывает на перераспределение минутного объема.

ИССЛЕДОВАНИЯ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ОТЕКЕ ЛЕГКИХ

I. Данные к патомеханизму возникновения отека легких, вызванного гипероксией

Э. МАКЛАРИ, М. КЕЛЛНЕР, А. КАДАР, А. Г. Б. КОВАЧ и Д. ГОТТЗЕГЕН

Было установлено, что в атмосфере с высоким давлением кислорода, повышение pO_2 уменьшает время выживания крыс и ускоряет развитие отека легких. Тканевую гиперкапнию можно выявить сперва в мышцах, затем в мозге и печени, а отек легких у большинства животных развивается лишь после этого.

Добавление CO_2 еще усиливает вредное действие высокого давления кислорода, и ускоряет возникновение нарушения легких.

ИССЛЕДОВАНИЯ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ОТЕКЕ ЛЕГКИХ

II. Действие дибензилина при отеке легких, вызванном гипероксией

Э. МАКЛАРИ, М. КЕЛЛНЕР, Г. Б. А. КОВАЧ и Д. ГОТТЗЕГЕН

Авторы исследовали действие дибензилина, блокирующего симпатические α-рецепторы, на развитие отека легких, вызванного высоким давлением кислорода.

Было установлено, что после предварительной дачи дибензилина увеличивается время выжывания животных, содержавшихся в атмосфере с высоким давлением кислорода, однако, дибензилин не препятствует развитию отека легких.

У животных, получивших дибензилин, наблюдалась незначительная тканевая гиперкапния, однако, без поражения легких.

После введения дибензилина, у животных, находящихся в атмосфере с высоким давлением кислорода, содержание CO_2 в тканях дальше не повысилось. Усиливающее нарушение легких действие сильной тканевой гиперкапнии, вызванной добавлением CO_2 к вдыхаемому кислороду, также было предотвращено дачей дибензилина.

На основании результатов авторы полагают, что тканевую гиперкапнию или повышенную активность симпатической нервной системы сами по себе нельзя считать ответственными за возникновение отека легких, вызванного гипероксией.

ИССЛЕДОВАНИЯ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ОТЕКЕ ЛЕГКИХ

III. Действие симпатиколитиков на адреналиновый отек легких у крыс

М. КЕЛЛНЕР, Э. МАКЛАРИ и А. Г. Б. КОВАЧ

Внутривенное введение адреналина крысам вызывало у всех животных судороги, и привело затем к смерти от тяжелого отека легких. Предварительная дача не предотвратила ни появления судорог, ни возникновения отека легких и, следовательно, не изменила времени выживания животных. Предварительная дача дибензилина у всех животных препятствовала возникновению судорог и развитию отека легких, и животные еще через 48 часов после введения адреналина находились в хорошем состоянии.

19—20 СЛУЧАЕВ НАСЛЕДОСТВЕННОЙ ТРИСОМИИ В СЕМЬЕ С МНОГОЧИСЛЕННЫМИ ВРОЖДЕННЫМИ АНОМАЛИЯМИ

Л. МОШОНЬИ, Д. ШУЛЕР, Е. АЧ и Ш. КИШШ

Авторы сообщают о семье, в которой в четырех поколениях наблюдались многочиселенные врожденные аномалии, чаще всего почек (поликистозные почки) и вывих бедра. На пяти членах указанной семьи были проведены определения хромосом. Кариограммы предположительного исходного лица, имевшего многочисленные аномалии развития, и его высокорослой, но в остальном здоровой дочери показали 46/47 мозаичную F-трисомию.

МЕХАНИЗМ ДЕЙСТВИЯ КОРТИКОСТЕРОИДОВ В ПРОФИЛАКТИКЕ И ТЕРАПИИ ШОКА. ДЕЙСТВИЕ ПРЕДНИЗОЛОНА В АДРЕНАЛИНОВОМ И НОРАДРЕНАЛИНОВОМ ШОКЕ

Д. САБО, Э. ШАТОРИ и Г. ГАНТНЕР

У собак в необратимом шоке, вызванном вливанием норадреналина, феноксибензамин в дозе 0,5 мг на кг веса тела оказывает выраженное защитное действие. Половина животных выживает после шока. Большая доза преднизолона не имеет терапевтического или защитного действия, а даже уменьшает выживаемость животных приблизительно на половину и повышает прессорное и вызывающее гемоконцентрацию действия норадреналина. По мнению авторов предположение, согласно которому профилактическое или терапевтическое действие кортикостероидов основывается на блокаде адренергических концевых аппаратов, не является вероятным.

ОСТРАЯ НЕДОСТАТОЧНОСТЬ ПОЧЕК, ВЫЗВАННАЯ У БОДРСТВУЮЩИХ СОБАК

И. ТАРАБА, Э. БЕНЕДЕК, Л. МОЛЬНАР и Л. ШТИАСНИ

1. У 13 собак с одной почкой авторы проводили безболевое ишемизирование почек на протяжении двух часов. 10 из 13 животных погибли с симптомами уремии, а трое собак жили еще после 14 дневного периода наблюдения.

2. При оценке результатов лабораторного и гистологического исследования погибших и выживших животных можно установить, что симптомы этой патологической картины в общем и целом идентичны с симптомами острой недостаточности почек у человека.

3. На основе полученных результатов и прежних сообщений авторы предполагают, что в возникновении болезни участвует рефлекторный процесс, переключающийся в центральной нервной системе и вызывающий в почках продолжительную ишемию.

ДЕЙСТВИЕ ИШЕМИИ НА СЕКРЕЦИЮ КОРТИКОСТЕРОНА КОРОЙ НАДПОЧЕЧНИКОВ У КРЫС

М. ДАВИД и К. КОВАЧ

Авторы на протяжении различного времени прекратили у крыс кровообращение в надпочечниках и исследовали, какие изменения вызывает местная ишемия в тканях, в весе, в кровотоке надпочечников и в секреции кортикостерона in vivo.

Было установлено, что двухчасовое лигирование надпочечниковых кровеносных сосудов вызывает в исследуемом органе обширный некроз. Выделение кортикостерона прекращается. Лигирование артерий в течение часа вызывает в корковом слое очаговые инфаркты. Понижается также секреция кортикостерона. Введением АКТГ не удалось повысить выделения кортикостерона. Это указывает на то, что клетки коры надпочечников, вследствие ишемии, утратили свою чувствительность в отношении стимулирующего действия АКТГ. После лигирования надпочечников в течение 30 минут некрозов не наблюдалось, но секреция кортикостерона уменьшилось и в таком случае.

Результаты исследований указывают на то, что в патологических условиях на выделение кортикостерона, помимо АКТГ, влияет также снабжение надпочечников кислородом.

НЕДОСТАТОЧНОСТЬ ПОЧЕК, ВЫЗВАННАЯ У КРОЛИКОВ ВЛИВАНИЕМ УРАТА

Исследование роли уратов в деле возникновения шоковой почечной недостаточности в экспериментах на животных

Д. БОДА, Й. ХАРИ и Д. СИНАИ

Согласно результатам опытов на животных быстрое выделение парентерально введенной мочевой кислоты вызывает в почках накопление уратов, их выпадение в канальцах и вторичное тяжелое нарушение почек.

При умеренном шоке вследствие кратковременного лигирования конечностей в значительной мере повышается чувствительность организма в отношении вредного действия урата на почки.

Вышеприведенные данные подкрепляют предположение, согласно которому склонность человеческого организма к шоковой недостаточности почек объясняется характерными для человека особенностями обмена мочевой кислоты.

ИССЛЕДОВАНИЕ АНТИДИУРЕТИЧЕСКОГО ГОРМОНА

Й. ХАРВАТ и В. ХОЛЕЧЕК

Метод Джеффера для определения АДГ в крови авторы модифицировали для выявления 1 микроединицы АДГ в 1 мл плазмы.

У нормальных, хорошо гидрированных людей, в крови не удалось выявить антидиуретической активности. Функциональная способность нейрогипофиза может быть изучена при помощи внутривенного введения 20—25 мл 10-процентного раствора поваренной соли. После этого концентрация АДГ повышается в плазме в пределах 20 минут до 15—30 мкг/мл. Подобную реакцию можно получить при помощи ингаляции амилнитрита. Амфетамин и атропин вызывают торможение выделения АДГ даже после стимулирования осморецепторов посредством повышения осмолярности. Следовательно амфетамин можно использовать при лечении первичного гиперадиуретизма, а подчас даже при лечении предменструальной напряженности.

Энзиматическая инактивация АДГ имеет значение в некоторых случаях вторичного гиперадиуретизма. У мужчин и у небеременных женщин нельзя выявить активности вазопрессиназы в крови. В случае беременности такая активность начинается к концу второго месяца и достигает своего максимума на седьмой месяц беременности и резко падает после родов. Вазопрессиназа происходит из плаценты. При токсемии беременных, однако, ее выделение недостаточное, в результате чего содержание АДГ в крови начинает повышаться.

При тяжелой недостаточности печени вазопрессиназная активность печени понижается. При отсутствии лечения микседемы наблюдается иногда некоторое повышение содержания АДГ в крови, вызванное недостаточной инактивации АДГ в периферических тканях.

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IRON METABOLISM AND ANAEMIA IN SYSTEMIC LUPUS ERYTHEMATOSUS AND RHEUMATOID ARTHRITIS

By

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(Received May 6, 1966)

Studies with ^{59}Fe have shown iron metabolism to be considerably increased in SLE and rheumatoid arthritis. ^{59}Fe utilization was decreased in 7 out of 11 patients with SLE and in 7 patients with rheumatoid arthritis. Increased activity was found over the liver and the spleen, where the iron is stored probably in the form of a complex not giving the Prussian blue reaction. The anaemia is attributed to three factors: insufficient bone marrow activity, shortened life span of erythrocytes, and a moderate degree of iron deficiency.

Rheumatoid arthritis is associated with connective tissue changes which have much in common with those in collagenosis. Therefore, rheumatoid arthritis has recently been grouped with the other collagenoses such as systemic lupus erythematosus (SLE), scleroderma, periarteritis nodosa, thrombopenic purpura, and dermatomyositis. Clinically, all these diseases are characterized by a more or less severe anaemia.

Among the haematological alterations, anaemia is well-known in SLE, although opinions still differ concerning its origin [20, 1, 6, 8, 12, 13, 14]. According to HARGRAVES et al. as well as HEINIVAARA et al. it is caused by iron deficiency, whereas the majority of authors regard it as a consequence of some autoimmune haemolytic process [14, 6, 1, 20, 21]. Yet, judging from the data in the literature and our own experience, SLE anaemia accompanied by jaundice is a rare condition. However, neither an increase in the indirect serum bilirubin level nor the demonstration of antierythrocytic antibodies support the haemolytic origin and other factors must be responsible for such anaemias.

There is a large literature on the question of anaemia associated with rheumatoid arthritis. Several authors identified the mechanism of this anaemia with the processes observed during infection. Thus, the apparent iron deficiency in infectious disease is due to the increased iron turnover. Reticuloendothelial iron storage increases and iron utilization decreases. An essential fact is that iron turnover considerably increases while iron absorption remains normal. The absorbed iron is not, however, used for haemoglobin synthesis but stored in the RES. At the same time the life span of the erythrocytes is shortened [25, 17]. Similar events can be seen in rheumatoid arthritis, when enteral

iron absorption is normal but ^{59}Fe quickly disappears from blood, and the life span of the red corpuscles becomes moderately shortened [15, 24, 27, 4].

There is a diversity of opinion concerning the serum iron binding capacity. Some authors have found it to be decreased or varying with the stage and activity of the disease, while others observed no change [22, 23, 11, 18].

Clinical experiments as well as the studies of JEFFREY on bone marrow cultures indicate that iron deficiency also plays a role in the anaemia [16, 26]. However, iron treatment is only effective in cases of low rheumatic activity, while anaemia manifests itself in active and severe cases. Reticuloendothelial iron storage is not increased in every case. In the autopsy material of GARDNER et al. a double amount of iron was found in the spleen but mainly in cases treated with iron by the intramuscular or intravenous route [26, 10]. DIXON et al. demonstrated a considerable increase of plasma volume in rheumatoid arthritis and suggested that dilution of blood played a role in the anaemia [7].

In addition to these factors, a functional disturbance in the bone marrow should also be considered in both SLE and rheumatoid arthritis [9, 27, 2, 19]. Thus, it seemed worthwhile to study iron metabolism with ^{59}Fe in the above conditions.

Material and method

The clinical material consisted of 9 patients with SLE, 11 with rheumatoid arthritis, one patient with periarteritis nodosa and one with Sjögren's syndrome. The age of the patients, 14 females and 8 males, was between 25 and 60 years.

Most of the patients received no antianaemic treatment at the time of examination, i.e. no iron containing drug, glycocorticoid or blood transfusion was given. Three patients, whose condition made it necessary, received prednisolone; examination was carried out during this treatment. In addition to routine haematological studies, the serum iron level was estimated and a reticulocyte count was done. Bone marrow puncture was also performed on some occasions. Iron metabolism was studied with ^{59}Fe by a method described previously [5]. Ten to 15 μC of ^{59}Fe were added to the serum obtained from fasting patients. The mixture was then incubated for a short time and reinjected intravenously. Thereafter, the disappearance of ^{59}Fe from the plasma was checked at 1/4, 1/2, 1, 2, 3 and 4 hours after reinjection. Activity of the erythrocytes as well as the activity over the liver, spleen and bone marrow was measured after 1, 4, 8 and 12 days. The life span of the erythrocytes was determined by means of ^{51}Cr in 6 patients with SLE and 5 patients with rheumatoid arthritis. Labelling was carried out *in vitro*, according to the usual procedure, *viz.* 15 ml of whole blood were added to 5 ml of ACD (acid-citrate-dextrose) solution and the mixture was incubated with 60 μC of Na_2HCrO_4 at 37 °C for 15 minutes. 100 mg of ascorbic acid was mixed to the blood before intravenous reinjection. The value for the 24 hour sample was considered 100 per cent and this served as a basis of comparison for the activities found at intervals of 2 to 3 days. Blood samples were taken until their activity had decreased to 50 per cent. Measurements were carried out *in vivo* by means of a scintillation detector with collimated NaI crystals of 35 mm.

SLE was diagnosed on the basis of the clinical picture and the presence of repeatedly demonstrated LE cells, while rheumatoid arthritis on that of characteristic articular changes, X-ray findings and a positive Rose test. Cases showing signs of activity were selected for investigation.

Results

Results are shown in Tables I and II, as well as in Figures 1 to 4.

1. The SLE group, which included in addition the patient with Sjögren's syndrome and the one with periarteritis nodosa, was characterized by moderate anaemia in all patients but one.

The serum iron level averaged 80 ± 16.60 μg per 100 ml in 11 patients; it was lower than 80 μg per 100 ml in four patients. The average serum iron level in our healthy male and female material was 105 ± 18.55 μg per 100 ml, as estimated with Laurell's method.

The disappearance of ^{59}Fe from plasma was considerably accelerated in all but three cases. It averaged 46 ± 27.20 minutes, while the normal values ranged between 60 and 120 minutes.

The turnover of plasma iron, by which is meant the amount of iron passing through the plasma per unit of time, was markedly increased in 7 patients and moderately decreased in one. The average value was 1.35 ± 0.01 mg/kg/24 hours, while the normal range is from 0.45 to 0.75 mg/kg/24 hours.

$$\frac{0.693 \times 1440 \times \text{plasma iron}/\mu\text{g/ml}/ \times \text{plasma vol.(3 l)}}{\text{Plasma } ^{59}\text{Fe half clearance time (min)}} =$$

plasma iron turnover mg/kg/day.

The venous haematocrit was near normal in one patient who had been treated with corticosteroids for a long time and was now suffering from iatrogenic Cushing's syndrome. The average value was 38 ± 1.4 per cent.

^{59}Fe incorporation into erythrocytes was accelerated in two female patients (A. Zs.: 96%/5 days, and J. H.: 90% /4 days). It was normal in two further cases and considerably decreased in 7 patients (A. S. ♀: 39 %/12 days, J. P. ♂: 35 per cent/12 days, J. B. ♀: 55 per cent/8 days, T. G. ♀: 11 per cent/12 days, J. Cs. ♂: 25 per cent/14 days, G. V. ♀: 55 per cent/8 days and I. D. ♂: 60 per cent/12 days.) (The normal rate is between 75 and 95% per 7 to 12 days.)

On the second day of examination, in 7 cases the highest activity was found over the liver, which was followed by the sacrum and the spleen. It is known that in normal erythropoiesis the highest activity is found over the sacrum. It should be mentioned that not only in relation to each other were the measured activities pathological, but they failed to decrease at the expected rate even on the 12th day (see Fig. 4).

In view of the imperfect ^{59}Fe utilization, the bone marrow was examined in 7 patients. In addition to a moderate increase in the number of plasma cells, an elevation was observed in the immature basophilic erythrocytes while the proportions of the other cells were relatively normal. The reticulocyte count

Table I

Laboratory findings in SLE

Case No.	Patient	Sex	Serum iron level, μg/100 ml	Plasma iron clearance, min	Plasma iron turnover mg/kg/24 h	Hb g per 100 ml	Haematocrit, %	^{59}Fe incorporation into erythrocytes, per cent/day	Diagnosis	Treatment and remarks
1.	A. S.	F	92	28	1.64	10.5	37	39/12	LED	Aminopyrine
2.	G. V.	F	45	40	0.55	10.5	36	55/8	LED	
3.	I. P.	M	87	28	1.50	9.5	38	35/12	LED	Vitamins, Aminopyrine
4.	J. B.	F	70	85	0.30	11.0	39	55/8	Sjögren-syndrome	Vitamins
5.	T. G.	F	84	18	4.00	9.6	37	11/12	LED	—
6.	I. D.	M	64	85	0.45	10.7	38	60/12	LED	—
7.	J. Cs.	M	120	45	1.56	10.0	39	25/14	Periarteritis nodosa	Prednisolone
8.	A. Zs.	F	80	40	0.80	8.8	34	96/5	LED	Spontaneous remission
9.	M. K.	F	75	30	1.16	11.0	38	86/13	LED	Remission during prednisolone treatment
10.	I. H.	F	83	20	2.35	9.5	37	90/4	LED	During Chloroquine treatment
11.	S. J.	F	80	90	0.52	12.0	40	95/8	LED	Improved after prednisolone treatment
Mean and s. e.			80 ± 16.8	46 ± 27.2	1.35 ± 1.01	10.4 ± 0.81	38 ± 1.4			
Normal range and s. e.			105 ± 18.55	60— 120	0.45— 0.75	14— 16	44— 46	75 to 95/ 7—12 days		

was normal (from 0.6 to 1.2 per cent) in all cases, indicating a disturbance of maturation.

2. The results obtained in the patients with rheumatoid arthritis were as follows.

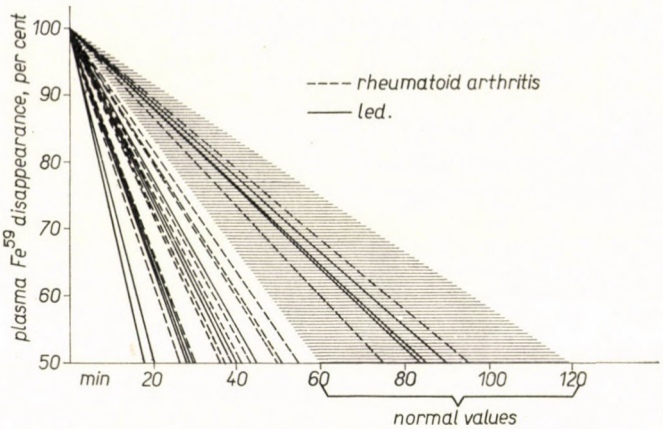

Fig. 1. Plasma ^{59}Fe clearance in rheumatoid arthritis and in systemic lupus erythematosus

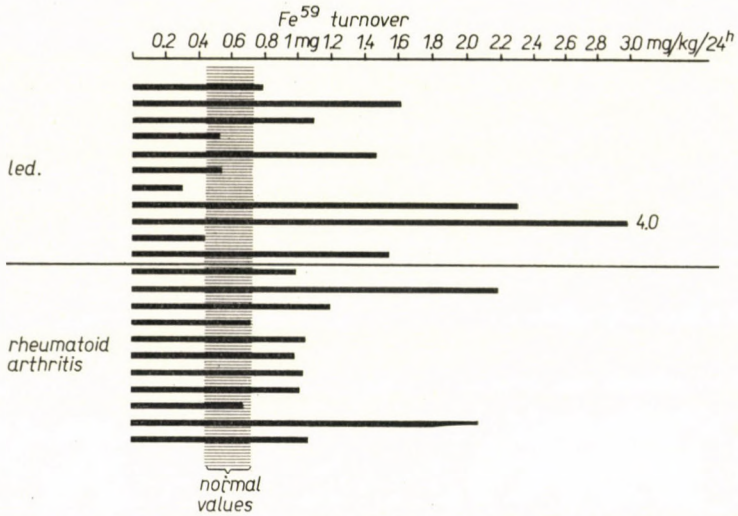

Fig. 2. Plasma iron turnover in rheumatoid arthritis and in systemic lupus erythematosus

No signs indicative of haemolytic anaemia were seen.

The value of the serum iron was within the normal range in 7 out of 11 cases, and diminished in four. The mean was 92 ± 20.82 μg per 100 ml (see Table II).

^{59}Fe disappearance from plasma was accelerated in 9 patients and normal in two, with a mean of 44 ± 13.30 (see Fig. 1).

Acta Medica Academiae Scientiarum Hungaricae 23, 1967

Table II

Laboratory findings in rheumatoid arthritis

Case No.	Patient	Sex	Serum iron level, μg/100 ml	Plasma iron clearance, min	Plasma iron turnover mg/kg/24 h	Hb g per 100 ml	Haematocrit, %	^{59}Fe incorporation into erythrocytes, per cent/day	Diagnosis	Treatment and remarks
1.	A. P.	M	90	27	1.00	12.2	42	75/13	Bechterew's disease	—
2.	J. T.	M	80	36	2.29	9.1	35	64/12	Bechterew's disease	—
3.	J. N.	F	77	45	1.20	11.0	37	60/12	Rheumatoid arthr.	Aminopyrine
4.	M. Zs.	F	108	50	0.72	13.3	44	98/8	Rheumatoid arthr	Prednisolone, butazolidine
5.	I. Sz.	F	54	42	1.10	10.7	37	58/12	Rheumatoid arthr.	—
6.	J. K.	F	100	75	1.00	11.6	43	65/8	Rheumatoid arthr.	Prednisolone, butazolidine
7.	J. B.	M	114	95	0.65	10.1	40	59/12	Rheumatoid arthr.	—
8.	J. R.	F	129	30	2.15	9.8	38	58/8	Rheumatoid arthr.	—
9.	I. B.	M	91	55	1.14	13.2	44	77/12	Rheumatoid arthr.	Prednisolone, Chloroquine
10.	J. Sch.	M	73	40	1.14	9.1	33	61/12	Rheumatoid arthr.	After Chloroquine butazolidine treatment
11.	F. B.	F	92	50	1.26	10.0	34	75/13	Rheumatoid arthr.	—
Mean and s. e.			92 ± 20.8	44 ± 13.3	1.04 ± 0.58	10.9 ± 1.16	39 ± 4.03			
Normal range and s. e.			105 ± 18.55	60— 120	0.45— 0.75	14— 16	44— 46	75 to 95 /7—12 days		

Plasma iron turnover was elevated in 9 patients, and normal in two cases. The mean was 1.04 ± 0.58 (see Table II).

^{59}Fe incorporation was somewhat delayed in 8 patients; 7 of these showed moderately decreased incorporation (J. B. ♂: 59 per cent/12 days, J. Sch. ♂: 61 per cent/12 days, J. T. ♂: 64 per cent/12 days, J. N. ♀: 60 per cent/12 days, I. Sz. ♀: 58 per cent/12 days, J. K. ♀: 65 per cent/8 days, and J. R. ♀: 58 per cent/8 days) (see Fig. 3).

Manifest anaemia was found in 8 patients, in three of them with marked iron deficiency (J. N. ♀, J. Sz. ♀, J. Sch. ♂). In three of the 11 patients the values for haemoglobin and haematocrit were near to normal. The mean haematocrit was 39 ± 4.03.

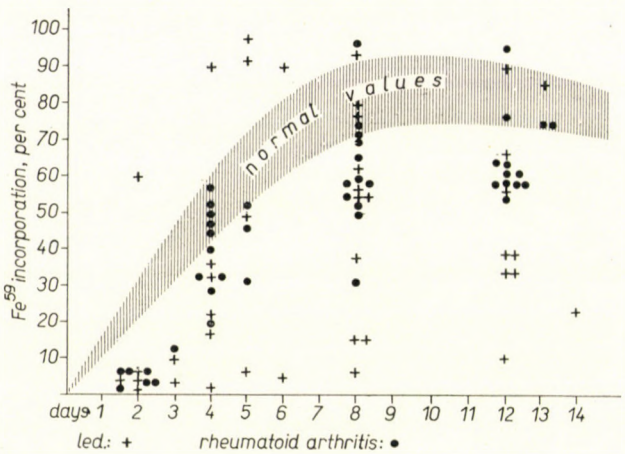

Fig. 3. ^{59}Fe utilization in rheumatoid arthritis and in systemic lupus erythematosus

Activity was increased over the liver in 8 out of the 11 patients, and over the spleen in one patient. There was a moderate difference between the patients with rheumatoid arthritis and those with SLE, as in the former a larger portion of the ^{59}Fe was incorporated into the erythrocytes and a smaller part in the storing organs, than in the SLE group (see Table III and Fig. 4).

The life span of erythrocytes was moderately shortened (between 23 and 27 days) in 6 patients with SLE and in 5 with rheumatoid arthritis. (I. D. ♂: 25 days, M. K. ♀: 27 days, A. S. ♀: 24 days, I. P. ♂: 25 days, T. G. ♀: 23 days, A. Zs. ♀: 25 days, A. P. ♂: 24 days, J. T. ♂: 25 days, M. Zs. ♀: 27 days, J. R. ♀: 24 days, F. B. ♀: 24 days). Hyperbilirubinaemia was absent in all. Fig. 5 shows the life-span curves which deviate somewhat from normal. Activity over the liver and the spleen were normal. (In patient I. P., the examination was made after splenectomy.)

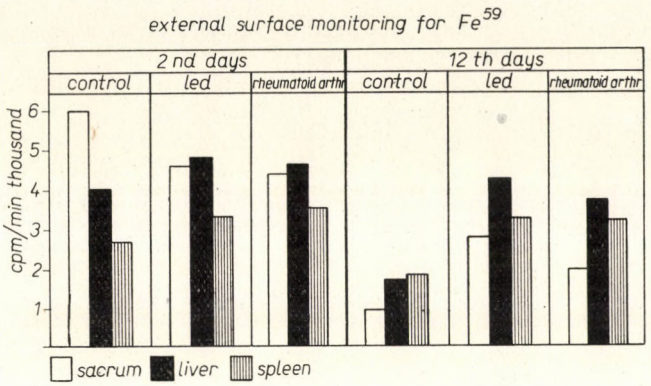

Fig. 4. External surface monitoring over sacrum, liver and spleen

Table III

Highest radioactivity	Control group	LED	Rheumatoid arthritis
Second day			
Sacrum	25	4	2
Liver	—	7	8
Spleen	—	—	1
Twelfth day			
Sacrum	—	—	—
Liver	25	11	9
Spleen	—	—	2

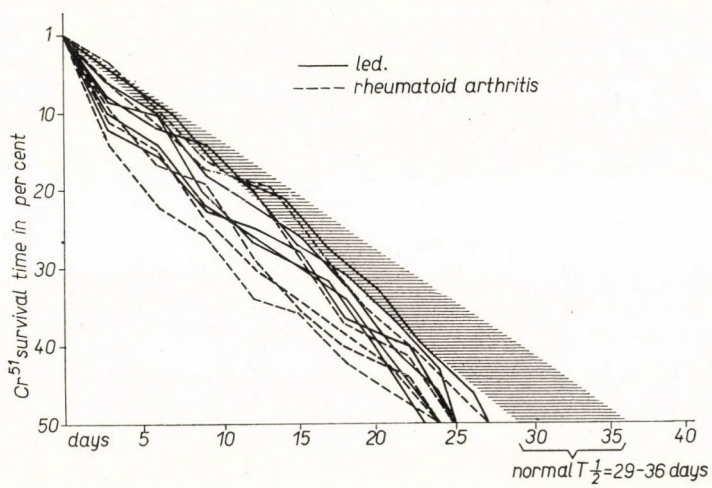

Fig. 5. ^{51}Cr labelled erythrocytes survival in rheumatoid arthritis and in systemic lupus erythematosus

Discussion

The results have shown that both in SLE and rheumatoid arthritis iron metabolism is considerably increased. In the majority of patients this was due not only to iron deficiency, as the serum iron level deviated but slightly from the normal. This agrees well with the observation that, in spite of normal iron absorption, oral iron treatment fails to influence this form of anaemia [15, 24]. Haemolytic anaemia is relatively rare and, although it is associated with a shortened life span of erythrocytes, the severe anaemia seen in SLE cannot be explained on this basis. In the majority of SLE patients, iron utilization is decreased. Radioactivity over various organs differed from normal; a similar change may occur in aplastic anaemia and viral hepatitis. When ^{59}Fe incorporation into erythrocytes is normal or somewhat delayed, but still reaches the usual rate of 75 to 90 per cent, there is a disturbance of iron metabolism, though in milder form. The difference between the activity measured over the liver and that over the bone marrow was slight and considerable activity was still present on the 12th day. All this indicates that some increased amount of absorbed iron did not serve the purpose of haemoglobin synthesis but was stored.

Plasma iron turnover was elevated in the majority of the patients with SLE and rheumatoid arthritis, which is at variance with the decreased iron incorporation. This might be in two ways. According to BOTHWELL et al. [3] the turnover of plasma iron is an indicator of the amount of iron used for erythropoiesis. They explain the marked differences in iron utilization in terms of total and effective erythropoiesis, assuming rapid destruction in the bone marrow of the immature erythrocytes which have taken up the iron. A second possibility would be the increased iron storage either in the labile form or as haemosiderin. GARDNER et al. [10], using the Prussian blue reaction, failed to see any increase in iron storage. On the other hand, there are protein-iron complexes which do not give this reaction but may still play a role in the pathological storage. It may thus be assumed that with decreasing activity the production of haemoglobin increases either spontaneously or in response to a drug, the erythron destruction in the bone marrow diminishes and the stored iron is used up. The enlargement of, and increased blood content in, liver and spleen are also responsible for the greater activity measured over these organs.

Thus, it may be concluded that, in addition to increased iron metabolism there is also a moderate degree of iron deficiency and increased iron storage in SLE and rheumatoid arthritis. The anaemia can be further accounted for by a shortened life span of the erythrocytes and mainly by the decreased bone marrow activity.

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RETROSPEKTIVES STUDIUM ÜBER DIE BEHANDLUNG DER DEPRESSION IN LONDON UND BUDAPEST

VORLÄUFIGE MITTEILUNG

Von

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(Eingegangen am 9. November (1966)

Voriges Jahr haben wir mit derselben Methode einen später zu bestimmenden Teil des Krankenmaterials des Maudsley Hospitals und der Budapester Psychiatrischen Klinik mit dem Ziel aufgearbeitet, daß wir die Behandlung der Depression objektiv verwerten können. Bei der Materialsammlung haben wir auch den Gesichtspunkt gehabt, daß wir eine Möglichkeit für den Vergleich eventueller ethnischer und sozialer Unterschiede haben.

I. Fragestellung

1. Besteht in der Reaktion Depressiver auf Elektroschocks oder Medikamente eine signifikante Geschlechtsdifferenz?

2. Wie sind die Behandlungserfolge beim Vergleich von Elektroschocks mit trizyklischen Antidepressiva (z. B. Imipramin) und MAO-Hemmern (vorwiegend Phenelzin) gegenüber nicht behandelten Depressionen?

3. Welche klinischen Merkmale korrelieren mit einer Therapieresistenz?

II. Methode

Wir verarbeiteten 910 Krankengeschichten depressiver Patienten, welche zwischen 1957 und 1963 ins Maudsley Hospital und Bethlem Royal Hospital in London aufgenommen wurden, und in derselben Periode 241 Krankengeschichten der Psychiatrischen Klinik Budapest. Informationen wurden gesammelt über *a)* Anamnese, *b)* prämorbide Persönlichkeit, *c)* vorausgegangene mentale und somatische Krankheiten, *d)* den somatischen und psychiatrischen Zustand bei der Aufnahme, *e)* Symptomatologie, *f)* Details der Behandlung, angegeben in Zeitdauer und Dosen, *g)* Reaktion auf die Behandlung nach 12 Monaten, *h)* allgemeine Veränderungen nach 4 Wochen und bei der Entlassung, *i)* follow up.

Die Selektion der Patienten geschah nach folgenden Kriterien:

a) Die Depression mußte die primäre Krankheit sein.
b) Es mußte sich um eine frisch aufgenommene Depression handeln.
c) Minimaldauer der Behandlung 2 Wochen.
d) Minimaldosierung für Imipramin 150 mg, für Phenelzin 45 mg.

III. Vorläufige Resultate

Die Patienten, welche Elektroschocktherapie erhielten, waren signifikant schwerer depressiv als die medikamentös behandelten. Patienten ohne somatische Behandlung waren deutlich weniger depressiv als die anderen und konn-

ten somit weder in London noch in Budapest zum Vergleich herangezogen werden.

a) *Die Frage einer Geschlechtsdifferenz in der Reaktion auf die Behandlung*

Das »Allgemein-Rating» über 4 Wochen zeigte in London, daß beim Elektroschock die 79 Männer, welche 166 Frauen gegenübergestellt wurden, auf dem 5%-Niveau signifikant besser auf die Behandlung ansprachen, als die Frauen; in Budapest haben wir das nicht bemerkt. In den übrigen Behandlungsgruppen fanden sich keine Differenzen.

Anhand des zweiten Erfolgskriteriums, nämlich der Klinik-Entlassungsraten über 5, 8, 12, 24, 39 und 52 Wochen zeigte sich gerade der umgekehrte Befund in London. Frauen wurden innerhalb der ersten 8 Wochen etwas

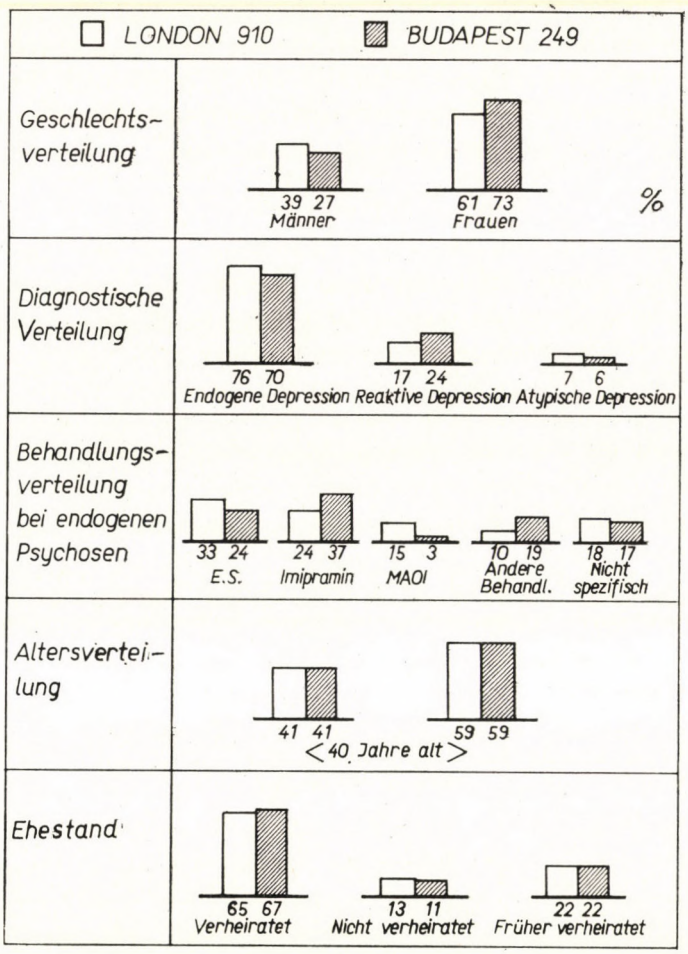

Fig. 1

häufiger nach Elektroschockbehandlungen aus der Klinik entlassen als Männer; nach 8 Wochen war der Unterschied auf dem 1%-Niveau signifikant, eliminierte sich aber später wieder völlig. Unter Imipramin, MAO-Hemmern und Patienten ohne somatische Behandlungen zeigten sich hingegen keine signifikanten Geschlechtsdifferenzen. Ähnlich war die Situation in Budapest.

b) *Vergleich der verschiedenen Behandlungsverfahren*

Als drittes Erfolgskriterium kann berücksichtigt werden, wieviele Erstbehandlungen innerhalb 5 bzw. 8 Wochen durch andere Behandlungsverfahren ersetzt werden mußten. Es zeigte sich dabei, daß innerhalb von 8 Wochen 21,6% der Elektroschockfälle in London und 30,5% in Budapest, 26,5% der Imipraminfälle in London und 27,0% in Budapest und von den MAO-Hemmer-Behandlungen 31,0% in London und fast alle in Budapest durch andere Verfahren ersetzt werden mußten. Auch diese Zahlen zeigen, daß der ES am seltensten durch andere Behandlungen ersetzt werden mußte, und daß die MAO-Hemmer am häufigsten abgesetzt wurden. Die Unterschiede zwischen den einzelnen Gruppen sind allerdings statistisch nicht signifikant, stimmen aber in ihrer Tendenz mit den anderen Resultaten überein.

c) *Therapieresistenz*

Eine weitere Untersuchung galt der Frage, ob klinische Merkmale gefunden werden können, welche mit einer gewissen Therapieresistenz korreliert sind. (Das haben wir in Budapest nicht festgestellt.) Zuerst wurden Kranke, die 4 Wochen nach Behandlungsbeginn noch keinerlei Besserung zeigten, den übrigen gegenübergestellt. Untersucht wurden folgende klinische Merkmale: Geschlecht, Schwere der depressiven Stimmung, nosologische Diagnose, soziale Anfälligkeit der prämorbiden Persönlichkeit, phasenauslösende Faktoren, Phasenzahl, Zivilstand, Beruf, Alter und Ersterkrankungsalter. In der Imipramin-Gruppe fanden sich keine signifikanten Beziehungen. Hingegen wurde in der Elektroschockgruppe folgendes deutlich: unter den Therapieresistenten häuften sich signifikant Kranke, die früher manisch erkrankt waren und vor dem 20. Altersjahr die erste Phase aufwiesen. Die Zahlen waren auf dem 0.1%-Niveau signifikant. Auf dem 5%-Niveau häuften sich ferner unter den Therapieresistenten die differenzierten Berufe (professionals).

IV. Diskussion

1. Unsere Untersuchungen ergaben keine Anhaltspunkte für eine Geschlechtsdifferenz der Ansprechbarkeit auf antidepressive Behandlungen. Lediglich bei ECT ließen sich anhand von 2 verschiedenen Erfolgskriterien Differenzen nachweisen; doch waren diese Resultate gerade entgegengesetzt, so daß sie keine Schlüsse zuließen.

2. Ein Vergleich verschiedener Behandlungsverfahren in einer retrospektiven Studie trägt viele Unsicherheiten in sich. So mußten wir z. B. in London eine Gruppe von 107 endogen Depressiven, die keine somatische Behandlung erhalten hatten, ausschließen, weil diese Kranken weniger schwer depressiv waren als die übrigen. Ein Vergleich war nur zwischen Elektroschocks, Imipramin und Phenelzin möglich. Es zeigte sich dabei eine gute Übereinstimmung bei den Erfolgskriterien. Es war festzustellen, daß das Phenelzin den beiden anderen Therapien signifikant unterlegen war. Der Vergleich zwischen ECT und Imipramin war schwieriger. Anhand der Entlassungsraten zeigte sich kein signifikanter Unterschied, hingegen war mit ES in den ersten Wochen eine etwas raschere Besserung zu erzielen.

Aus diesen Resultaten kann folgendes geschlossen werden:

a) Phenelzin erweist sich als wenig oder kaum wirksam bei der Behandlung von endogenen Depressionen;

b) Der ES wirkt etwas rascher als Imipramin, was sich aber in den Entlassungsraten nicht faßbar äußert.

3. Die Analyse der Therapieresistenz anhand von 2 Kriterien ergab folgendes:

a) Gemessen an einem »Allgemein-Rating« sprachen auf ES ältere Kranke, wiederholt Erkrankte und schwer Depressive schlechter an, als jüngere, erstmalig Erkrankte und leicht Depressive.

b) Hospitalisiert waren 6 Monate nach Behandlungsbeginn vor allem Kranke, die früher manische Phasen durchgemacht hatten, oder deren erste Attacke vor dem 20. Altersjahr erfolgte und die zu höheren Berufsschichten gehörten.

c) Ohne prognostischen Einfluß sind: Geschlecht, soziale Anfälligkeit der prämorbiden Persönlichkeit, auslösende Faktoren und Zivilstand. Diese Aussage muß allerdings eingeschränkt werden, da die genannten Merkmale noch nicht in jeder Richtung bezüglich ihres Einflusses untersucht sind. Bekannt ist z. B., daß verheiratete Patienten früher entlassen werden können, als ledige (PFEIFFER und BENTE 1961, ANGST 1965).

4. Auf Grund der bisherigen Erfahrungen scheinen uns die angewandten Erfolgskriterien zur retrospektiven Beurteilung antidepressiver Behandlungen nützlich. Am objektivsten ist dabei das Kriterium der Klinikentlassung. Es hat aber wiederum den Nachteil, daß es von vielen Variablen abhängig ist, da ja die Entlassung nicht immer nur auf Grund einer Besserung des Krankheitsbildes stattfindet. Es bietet jedoch keine Schwierigkeit, derartige Variablen in die Untersuchung einzubeziehen.

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PROBLEMS OF DETERMINING THROMBOCYTE LIFE SPAN

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A method is described to measure the life span of platelets by the aid of ^{51}Cr. The amount of isotope bound to the thrombocytes is directly proportional to the thrombocyte content of the suspension and ^{51}Cr activity. Optimum temperature for labelling lies between 18 and 22 °C. In the first days activity of the thrombocyte suspensions decreases, as a rule exponentially, and gives a straight line with a half life of 3.5 to 4.5 days, if plotted semilogarithmically. Total life span was 9 to 11 days in both homologous-autologous and ABO compatible and incompatible suspensions. The real life span of the thrombocytes is, however, longer, namely 12 to 16 days.

Although the fate in the organism of the labelled thrombocytes is not clear and measurements *in vivo* fail to give uniform results, it is certain that the site of thrombocyte destruction is the spleen, liver and lungs.

The method, as a routine clinical procedure, may be of great help in the differential diagnosis of haemorrhagic diatheses of different origin.

DUKE (1911) was the first to attempt to determine the life span of blood platelets. He could follow reinjected thrombocytes for five days in the dog [10]. Since, however, the experiments were not performed under physiological conditions, their results cannot be considered reliable. The introduction of labelling techniques has then offered better possibilities.

Labelling of thrombocytes with ^{32}P was first employed by JULLIARD et al. (1952). This was followed by the use for this purpose of ^{14}C-tryptophan (UDENFRIEND and WISSBACH, 1954), ^{131}J (MORGAN et al., 1954), ^{51}Cr-chloride (ROBERTSON et al., 1954), ^{51}Cr-chromate (MORGAN et al., 1955), ^{35}S-sulphate, ^{35}S-methionine and ^{14}C-formate (ODELL et al., 1955), diisopropylfluoro-^{32}phosphate (LEEKSMA and COHEN, 1956), ^{35}S-taurine (TRUHAUT and CLANET, 1958) and colloidal ^{198}Au (MAUPIN and LOVERDO, 1959) [13, 31, 28, 29, 19, 26, 25, 14, 30, 18]. The large number of these experiments shows that, in spite of the possibilities inherent in the tracer technique, determination of the life span of blood platelets still meets with difficulties, the greatest being the problem of creating physiological conditions. This circumstance as well as the different physical and biological properties of the isotopes used were probably responsible for the discrepancies in the results of the various authors.

Among the methods so far employed it is that of AAS and GARDNER [1], using ^{51}Cr, which seems to be the most suitable for labelling blood platelets and preparing an appropriate thrombocyte suspension. The procedure is now widely used for clinical as well as for experimental purposes [1, 21, 22, 5, 3, 15,

Table I

Thrombocyte life span as estimated with radioactive isotopes

Author	Isotope used	Labelling	Species	Result day-hour	Year
JULLIARD et al. [13]	^{32}P	in vitro	human	2 h	1952
MUELLER [20]	^{32}P	in vitro	rabbit	2 h	1952
ODELL et al. [23]	^{14}C	in vitro	rat	4 d	1953
MORGAN et al. [19]	^{131}J	in vitro	rabbit	2 h	1954
ROBERTSON et al. [29]	$^{51}CrCl_3$	in vitro	rat	2 d	1954
ODELL et al. [24]	$^{14}C+^{35}S$	in vitro	rat	5—6 d	1954
ODELL et al. [26]	^{14}C	in vitro	rat	1—3 d	1955
ODELL et al. [25]	^{35}S	in vitro	rat	4—5 d	1955
DESAI et al. [9]	^{32}P	in vitro	human	4—5 d	1955
MORGAN et al. [19]	$Na_2{}^{51}CrO_4$	in vitro	rabbit	3—4 d	1955
LEEKSMA et al. [14]	$DF^{32}P$	in vitro	human	8—9 d	1956
REISNER et al. [28]	$Na_2{}^{51}CrO_4$	in vitro	human	5—8 d	1956
ADELSON et al. [2]	^{32}P	in vitro	human	7 d	1957
AAS et al. [1]	$Na_2{}^{51}CrO_4$	in vitro	human	9—11 d	1958
NAJEAN et al. [21]	$Na_2{}^{51}CrO_4$	in vitro	human	9—11 d	1959
COHEN et al. [8]	$Na_2{}^{51}CrO_4$	in vitro	human	9—11 d	1961
BARTA et al. [5]	$Na_2{}^{51}CrO_4$	in vitro	human	9—11 d	1963
NAJEAN et al. [22]	$Na_2{}^{51}CrO_4$	in vitro	human	9—11 d	1963
AATER et al. [3]	$Na_2{}^{51}Cro_4$	in vitro	human	8—10 d	1964
MARCUS et al. [15]	$Na_2{}^{51}Cro_4$	in vitro	human	9—11 d	1965
BALDINI [4]	$Na_2{}^{51}Cro_4$	in vitro	human	9—11 d	1966
GARDNER et al. [11]	$Na_2{}^{51}CrO_4$	in vitro	human	9—11 d	1966

4, 11]. Essentially, this was the method used in the present experiments. Some minor modifications were, however, necessitated by the special conditions in our laboratory (see Table I).

Preparation and labelling of thrombocyte suspensions

The blood was collected in bottles used for storing bank blood. Before use the bottles were siliconized and the needles collodionized. Other parts of the equipment consisted of PVC tubing. The bottles were sterilized with dry heat and filled with a one per solution of Na_2EDTA (disodium ethylendiamine tetraacetate dihydrate) amounting to one-tenth of the volume of blood to be withdrawn, which was generally 20 ml, made isotonic with 0.7 to 0.8 per cent of sodium chloride adjusted to pH 7.2. The first 5 to 10 ml of blood that was obtained after inserting the needle was discarded because it inevitably contained tissue fluids. Thereafter, 20 250 ml of blood was collected in a bottle chilled to +4 °C. After centrifuging the whole blood at 1000 rpm for 10 minutes at +4 °C, the thrombocyte-rich plasma was transferred to siliconized chilled sterile test tubes and, in order to separate the erythrocytes still present, spinned at 2000 rpm for five minutes. After transfer into fresh siliconized tubes, the plasma was centri-

fuged at 3000 rpm for 30 minutes at +4 °C, which led to the appearance of a cream-like thrombocyte sediment. The supernatant plasma, containing hardly any blood platelets, was collected in a sterile flask. The thrombocyte sediment was resuspended in ten ml of plasma and 400 μC of $Na_2{}^{51}CrO_4$ were added. ^{51}Cr supplied by the Hungarian Atomic Energy Committee was sterilized by dry heat before use. Preparations of high specific activity are only suitable for these examinations, partly because of their alkalinity and partly because *in vitro* the free Cr ions may damage the blood platelets. The lowest specific activity to be used is 1 mC/ml/0.5 mg of Cr.

After addition of the isotope, platelet suspension was left standing at room temperature for 30 minutes. Thereafter, 100 mg of ascorbic acid was added in order to reduce the non-bound six-valent Cr ions to the trivalent form. This procedure prevented the erythrocytes from taking up *in vivo* ^{51}Cr during reinjection of the suspension. A further incubation at +4 °C for 10 minutes was followed by centrifuging the mixture at 3000 rpm for 30 minutes. Then the supernatant was discarded and the platelet sediment resuspended in a mixture of 8 ml of plasma, set aside earlier, and 3 ml of EDTA. Of this mixture, ten ml were reinjected intravenously with a siliconized glass syringe and the remainder used for determination of the thrombocyte count under the phase contrast microscope. The suspensions contained 1.5 to 3 million platelets per cu. mm.

ASTER and JANDL [3] called attention to the considerable number of platelets which could not be recovered from a platelet suspension prepared with EDTA within 24 hrs of administration, and attributed this loss to a harmful effect of EDTA. They observed that in suspensions prepared with EDTA the platelets underwent morphological changes, losing their disc form and becoming spherical. By binding their Mg ions, EDTA has an untoward effect on the metabolism of the platelets. The quoted authors suggested the use of a modified ACD solution (0.085 M trisodium citrate, 0.065 M citric acid and 2 per cent dextrose, sterilized by Seitz filtration) which did not damage the platelets and allowed an average 72 per cent of the labelled platelets of the injected thrombocyte suspension to be recovered in the circulation. When EDTA is used, an average of 28 per cent of the thrombocytes could only be recovered. In our experiments the modified ACD solution failed to give such good results, the chief difficulty being an aggregation of platelets. Therefore, preference was given to EDTA.

Results

The biological properties of blood platelet suspensions

When suspensions of different platelet counts were incubated with a solution containing various amounts of ^{51}Cr, the results obtained were identical with those described by AAS and GARDNER, the rate of isotope uptake being in direct proportion to both the platelet count and the specific activity of the isotope [1]. Labelling is best done at a temperature of 18 to 22 °C; under these conditions about 2 to 4 per cent of the added isotope will be bound to the platelets. Since the labelled thrombocyte suspension was not washed, the binding of a certain number of trivalent ^{51}Cr ions to the plasma proteins had also to be taken into account. Repeated checking of the thrombocyte-poor plasma

revealed decreased activity, in agreement with the experience gained in plasma protein clearance studies. In addition, activity of the erythrocyte suspension was also low. Activity of the whole blood after reinjection of the labelled thrombocyte suspension was about twice that of the platelet suspension prepared from the recipient's blood (Fig. 3).

Peak activity of platelets was observed 20 to 24 hours after the reinjection. Then a fall ensued, followed after 9 to 11 days by the complete disappear-

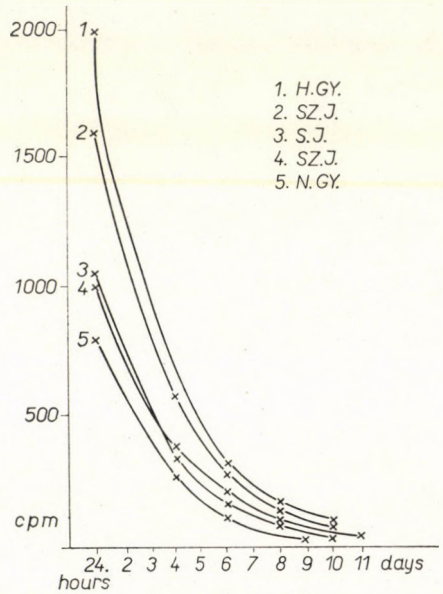

Fig. 1. ^{51}Cr activity in $Na_2{}^{51}CrO_4$ labelled platelets in five normal volunteer recipients

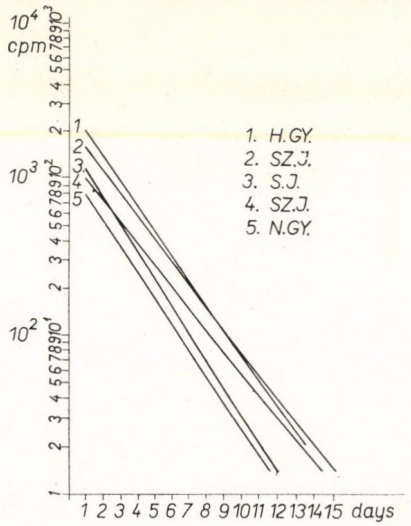

Fig. 2. ^{51}Cr activity in $Na_2{}^{51}CrO_4$ labelled platelets in five normal volunteer recipients. Semilogarithmic scale

ance of activity. In accordance with the data in the literature, this period represented the thrombocytes' life span [14, 27, 1] although it might have represented a value just as relative as the life span of the erythrocytes measured by means of ^{51}Cr. Data in the literature namely show that Cr ions are liberated from platelets just as from erythrocytes at a daily rate as high as ten per cent [18, 21]. The exact determination of the life span is further hindered by the difficulty in measuring low activities. It is also to be taken into account that the vitality of the thrombocytes decreases in the course of preparation. On the basis of all these considerations one is justified in assuming that the real life span of the blood platelets is longer, *i.e.* 12 to 16 days. This assumption is supported by our experiments on blood platelet suspensions treated with a 1.5 per cent solution of Nipagin (p-oxybenzoic acid methyl ester). These suspensions were prepared of a triple amount of blood (700 ml) and contained 2.5 to 3 million platelets per cu. mm. When these suspensions were injected to ABO

and Rh compatible control subjects, platelet suspensions of significant activity could be prepared for 13 to 15 days. The results obtained with other methods and in different species are summarized in Table I. Graphic representation of the activity values of the platelet suspensions prepared every, or every other, day does not always yield identical curves. Their shape depends on the extent

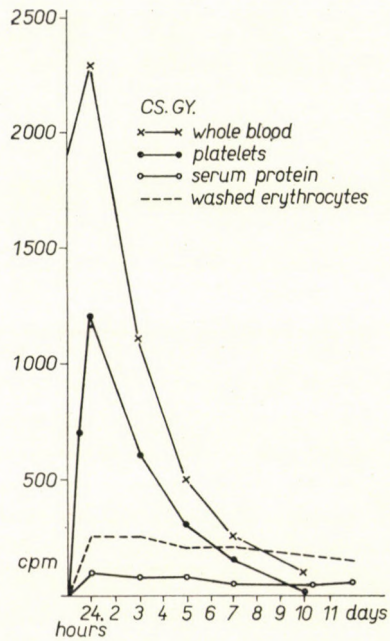

Fig. 3. ^{51}Cr activity in $Na_2{}^{51}CrO_4$ labelled platelets and separated blood components after infusion in a normal volunteer recipient

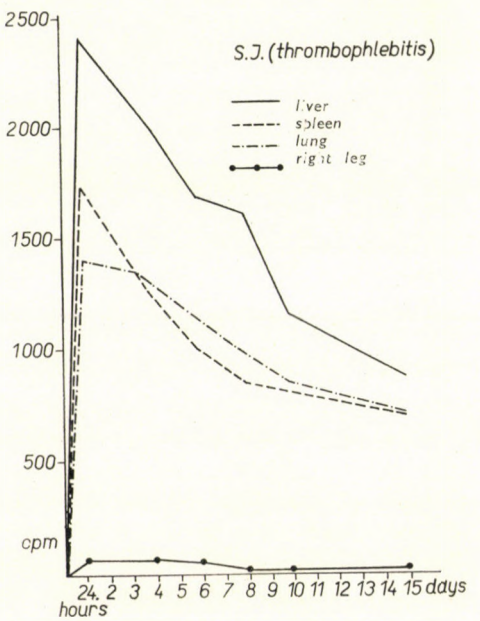

Fig. 4. Organ and right leg radioactivity after transfusion of ^{51}Cr labelled platelets in a patient with thrombophlebitis

of platelet injury during labelling, on the mode of plotting and, under pathological conditions, on the speed of destruction and regeneration of the platelets. A straight arithmetical curve means that every thrombocyte is viable for a definite period of time. On the other hand, an exponential curve is indicative of the accidental influence of biological factors on the life span of the platelets. Taking a 24-hour-old suspension, which has the highest activity, for 100 per cent, all other values can be expressed in percentages of this. Thus a curve is obtained which can be used for calculation under normal conditions. With an improved procedure AAS and GARDNER [1] obtained a nearly straight line. NAJEAN et al. [18, 21], allowing for ten per cent variations, obtained a curve of linearly decreasing activity. In the course of our investigations we met with both types of curves, an experience shared by other authors [8]. The curve probably consists of two parts, the initial being exponential and the second arithmetic. In pathological cases, however, the curve is entirely of exponential

character. Although the question has not completely been clarified, it can be established that through the distribution of blood platelets, the immunological and blood-clotting processes of the organism can change the number of labelled thrombocytes in circulation. A semilogarithmic diagram shows the half-life span to be 3.5 to 4.5 days in healthy controls, which partly agrees with the calculations of COHEN et al. [8]. (See Figs 1, 2.)

The fate of the labelled platelets in the organism still remains to be answered. It is known from animal experiments that the liver, spleen, lungs and bone marrow contain large numbers of labelled thrombocytes [19, 29, 17, 16]. The blood platelets which have disappeared from the blood are either phagocyted by the reticuloendothelial system or stored in the endothelium of the blood vessels. CRONKITE [6] demonstrated by autoradiography the presence of thrombocytes in the vascular wall; later he found many labelled thrombocytes in the macrophage system of the spleen. However, in the thrombopenic rats used in those experiments the platelet activity curve was normal after the infusion of thrombocytes, and therefore a "thrombocyte hunger" of the endothelial tissue could not be demonstrated [7].

The life span of the thrombocytes is unaltered in congenital disturbances of blood clotting, provided the examination is made when the patient is in a compensated state. In the case of bleeding, however, a shortened life span is to be expected because of a loss of labelled thrombocytes. In thrombotic or thromboembolic processes activity *in vivo* is elevated with a minimum decrease of the life span (Fig. 4).

Activity of various organs *in vivo* such as the liver, spleen and lungs corroborates the results of the above animal experiments. Peak activity was found over the liver after 24 hours, while there was no marked difference in activity between the spleen and the lungs. Considering, however, that the liver is 5 to 8 times as large as the spleen and the lungs are also larger, the highest relative activity is found over the spleen, *i.e.* sequestration of the platelets takes place mainly in this organ. Continuous monitoring of the activity over various organs gives a double-peaked curve; the peaks correspond to sequestration due to damage during labelling, and to ageing of the thrombocytes, respectively. In most cases, however, the decrease in radioactivity is linear (Fig. 5).

The thrombocyte life span was determined in ten healthy subjects with autologous blood platelet suspensions, which, on the basis of immunobiological considerations, were preferred also in pathological cases. Differences were not seen even when ABO-Rh compatible homologous suspensions were used (ten examinations). In ten healthy control subjects experiments were also made with random, *i.e.* ABO incompatible thrombocyte suspensions. The results were identical with those obtained in the former experiments, the life span of the thrombocytes being between 9 and 11 days.

It is hardly more than a decade ago that successful experiments were made to measure the life span of thrombocytes. Although several questions are still unanswered, it is beyond doubt that labelling, especially with ^{51}Cr, is suitable for routine clinical investigations. This method, together with the data of a bone marrow study and the thrombocyte count, allows to decide

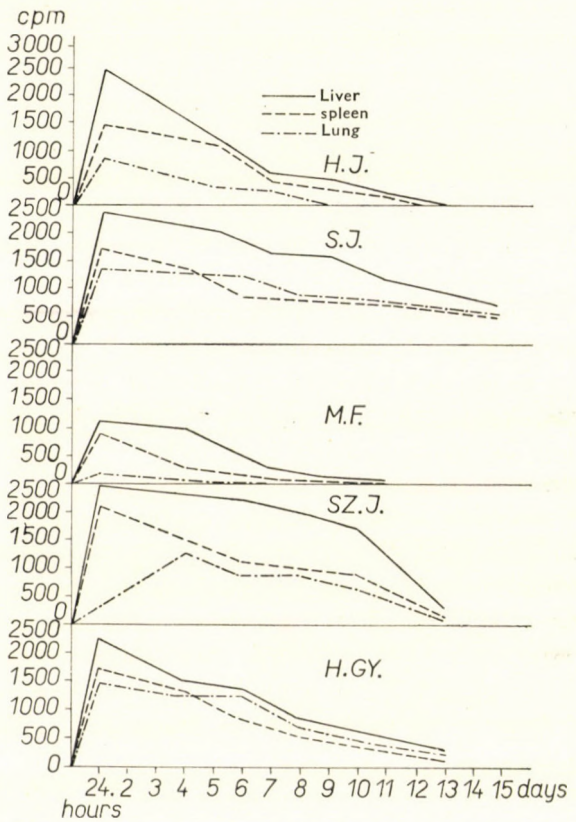

Fig. 5. Organ radioactivity after transfusion of ^{51}Cr labelled platelets to five normal subjects

whether diminished thrombocyte production or increased blood platelet destruction or both are responsible for a given haemorrhagic diathesis. It is even a help in the assessment of the pathogenesis in the so-called idiopathic diseases.

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PULMONARY OEDEMA

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Albino rats were given intravenously 2.5 per cent and 10 per cent solutions of glucose, and 0.9 per cent solution of sodium chloride, respectively, at a dose of 3 per cent of body weight, into the caudal vein. When this treatment was combined with the administration of 50 mU of vasopressin into the caudal vein and 30 minutes later of a similar dose intramuscularly, severe pulmonary oedema developed within 60 minutes. Administration of fluid without vasopressin and, conversely, of vasopressin without fluid, failed to induce oedema. Oedema is assumed to have been due to two factors, a diminution of osmotic pressure, and an increase of the permeability of pulmonary capillaries.

Acute pulmonary oedema can be induced by a variety of methods; according to Visher et al. (1956) and Korner (1953), it is the result of several factors acting simultaneously, which mutually promote the effect of one another. This is in accord with the observation that pulmonary oedema may accompany various pathological conditions (Luisada and Cardi, 1956) but it is difficult to establish which of the mechanisms in a given case is responsible for the accumulation of fluid in the alveoli. While studying renal function in rats we have become aware of the fact that experimentally induced moderate hypervolaemia, when accompanied by the administration of posterior pituitary extracts, led to death in most of the animals, with typical symptoms of pulmonary oedema. This observation led us to devote a more detailed study to the problem.

Methods

Experiments were performed on a total of 95 Wistar rats of either sex, weighing 170 to 300 g. The animals were anaesthetized with intraperitoneal Pentothal in a dose of 4 mg per 100 g. Through an abdominal midline incision both ureters were exposed and ligated near the bladder, in order to prevent the elimination of intravenously administered fluid. Following this procedure, the animals were arranged in 7 groups. In Groups 1, 2 and 3 a hypervolaemic state was induced by injecting different solutions into the caudal vein at a dose of 3 per cent of body weight. Group 1 received 2.5 per cent glucose solution (hyposmotic hypervolaemia); Group 2, 0.9 per cent NaCl solution (isosmotic hypervolaemia); Group 3 was given 10 per cent glucose solution (hyperosmotic hypervolaemia). Groups 4, 5 and 6 received the same doses of the same solutions with 50 mU of vasopressin (Pitressin, Parke-Davis). Thirty minutes later, further 50 mU of vasopressin dissolved in 0.2 ml of physiological saline was given intramuscularly in Groups 4, 5, 6. Animals of Group 7 after ligation of the ureters into the caudal vein received 50 mU of vasopressin in 0.5 ml of physiological saline, and 30 minutes later a similar dose intramuscularly.

Exactly 60 minutes after the injection of solutions the animals were killed with the intravenous injection of 0.5 ml of 3 M KCl. The thorax was opened and both lungs were re-

moved. The surface of the lungs was cleaned from blood, the lungs were weighed with an exactness of 1 mg. The left lung was fixed in 4 per cent formalin at 40 °C, the right one was weighed and then dried at 100 °C to constant weight. The lungs fixed in formalin were subjected to histological study. The sections were stained with haematoxylin-eosin, van Gieson's, Azan and resorcin-fuchsin according to Weigert. ECG recordings were made in all animals, first at the beginning of anaesthesia, then 10 minutes after the administration of solutions, and immediately before the animals were killed. Water contents of the lungs were expressed in per cent of total wet weight. The per cent ratio of wet weight of the lungs and body weight was also calculated. Oedema was designated by crosses, according to extent.

Results

Results are summarized in Table I. In Group 1 consisting of 10 animals pulmonary weight was 221 ± 24.5 g, while the wet weight of the lungs, 2.192 ± ± 0.600 g. The ratio of pulmonary to body weight averaged 1.0 ± 0.31, mean water content of lungs was 79.9 ± 1.36 per cent. In none of the animals could pulmonary oedema be found (Fig. 1).

Group 2 consisted of 12 animals. Their mean weight was 203 ± 35.6 g; pulmonary weight, 1.534 ± 0.343 g; the ratio lung/body 0.76 ± 0.21, the amount of water in the lungs 79.5 ± 1.38 per cent. No sign of pulmonary oedema could be revealed in the histological sections.

Table I

Group	Administered solution	Number of animals	Body weight g	Weight of lungs g	$\frac{\text{Lung weight}}{\text{body weight}} \cdot 100$	Fluid contents of lungs	Histology
1.	2.5 per cent glucose	10	±221 24.5	±2.192 0.600	±1.0 0.31	±79.7 1.36	— — —
2.	0.9 per cent NaCl	12	±203 35.6	±1.534 0.343	±0.76 0.21	±79.5 1.38	— — —
3.	10 per cent glucose	6	±200 28.1	±1.823 0.321	±0.93 0.24	±73.9 4.98	— — —
4.	2.5 per cent glucose +50 mU vasopressin i.v. (+50 mU i.m.)	16	±253 32.6	±2.145 0.512	±0.85 0.23	±78.6 6.4	+ + +
5.	0.9 per cent NaCl +50 mU vasopressin i.v. (+50 mU i.m.)	24	±217 48	±1.843 0.348	±0.87 0.22	±81.3 1.00	4 animals + + 20 animals + +
6.	10 per cent glucose +50 mU vasopressin i.v. (+50 mU i.m.)	12	±200 33	±1.839 0.281	±0.83 0.24	±72.2 8.10	2 animals + + 10 animals + + +
7.	50 mU vasopressin 0.5 ml of physiological saline i.v. (+50 mU i.m.)	15	±211 11	±2.010 0.360	±0.96 0.26	±74.0 5.00	— — —

In Group 3 of 6 animals mean body weight was 200 ± 28.1 g; wet weight of lungs, 1.83 ± 0.321 g; the ratio lung weight to body weight, 0.93 ± 0.24; water content of the lungs, 73.9 ± 4.98 per cent. Histological examination

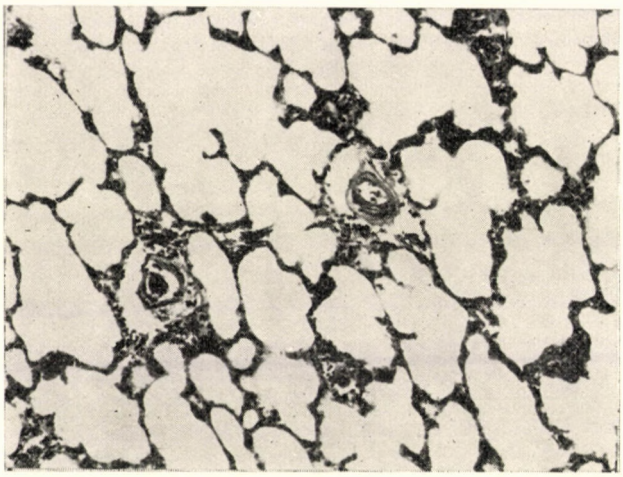

Fig. 1. No sign of oedema or perivascular fluid accumulation

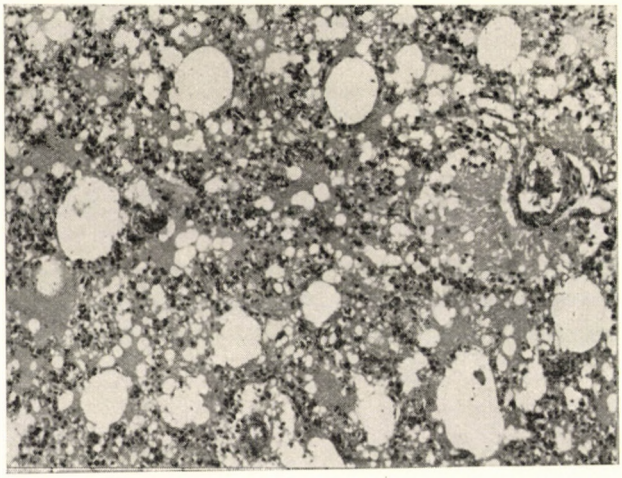

Fig. 2. Diffuse oedema and stagnation (Haematoxylin-eosin)

showed no pulmonary oedema. In Group 4 with 14 rats, body weight was 253 ± ± 32.6 g; pulmonary weight, 2.145 ± 0.512 g; the ratio of the two, 0.85 ± ± 0.23. The amount of water found in the lungs averaged 78.6 ± 6.4 per cent. Despite the fact that these values are very close to those in the first group, each of the animals showed histological signs of severe pulmonary oedema in

all parts of the left lung (Figs 2, 3 and 4). In Group 5 of 24 animals body weight was 217 ± 0.348 g; pulmonary weight, 1.843 ± 0.348 g, the ratio of the two, 0.87 ± 0.22. Pulmonary water content was 81.3 ± 1.00 per cent. The ratio body weight to lung weight was somewhat higher than in Group 2, the water content of the lungs was also slightly increased. The difference did not reach statistical significance. Histological observation revealed in 20 animals diffuse oedema (+++), while in the remaining 4 the oedema was severe but did not involve the whole lung (++).

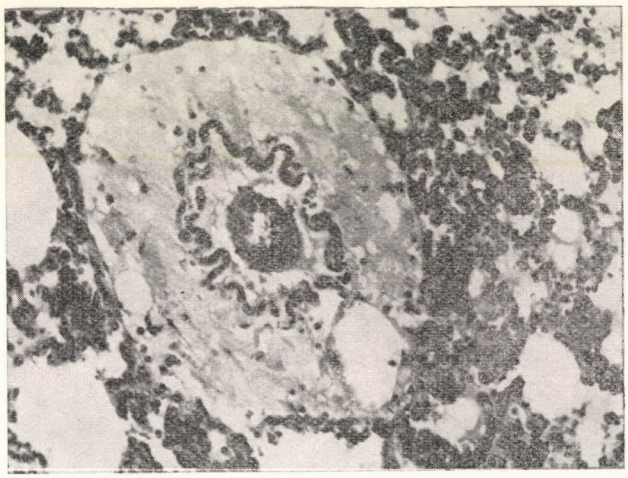

Fig. 3. Oedematous vessel walls. Heart muscle cells along the pulmonary vessels are separated from each other. Periadventitial space filled with homogeneously staining fluid. Haematoxylin-eosin

In Group 6 of 12 animals total body weight, pulmonary weight, the ratio lung weight/body weight and pulmonary water content were 220 ± 33.9 g, 1.893 ± 0.281 g, 0 83 ± 0.24 per cent and 72.2 ± 8.1 per cent, respectively. There was no statistically significant difference between Groups 5 and 2 in respect of these data. Histology revealed in 10 animals severe oedema involving the entire lung (+++) and in 2 animals moderate oedema (++).

In Group 7 with 15 animals body weight averaged 211 ± 11 g, wet lung weight was 2.010 ± 0.360 g, the ratio body weight to pulmonary weight, 0.96 ± 0.26, pulmonary water content, 74.0 ± 5.0 per cent. None of the animals showed histological signs of pulmonary oedema.

The results have shown that pulmonary oedema was present in all rats rendered hypervolaemic and treated simultaneously with vasopressin. Oedema developed within 60 minutes following the administration of fluid and involved in most cases the entire lung.

Irrespective of the method by which oedema of the lungs had been induced, the histological picture was similar. The alveoli were filled with fluid stain-

ing a homogeneous light red with haematoxylin-eosin and blue with Azan; it communicated with the fluid present in the periadventitial space of the capillaries (Figs 2 and 4). The wall of the precapillaries was oedematous, the endothelium swollen at sites; the internal and external lamina elastica stained intensively with resorcin-fuchsin, many fibres were loosened up or broken.

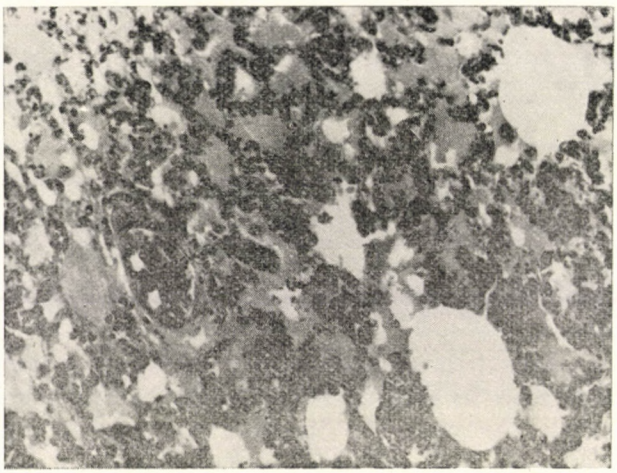

Fig. 4. Periadventitial fluid communicating with alveolar oedema. Haematoxylin-eosin

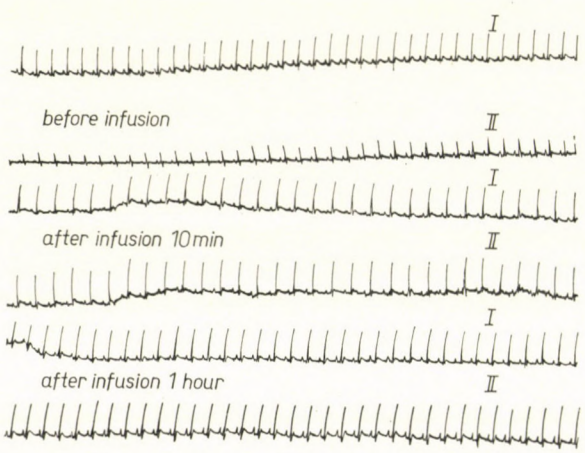

Fig. 5. Electrocardiogram of a rat with pulmonary oedema. Leads I and II

Cardiac muscle cells extending from the atrium along the pulmonary vein (CALABRESI 1962), were separated from one another and showed vacuolization. The periadventitial space was tightly filled with fluid staining a light red with haematoxylin-eosin and blue with Azan. A great number of small lymphatics, often with a valve, could be found in the periadventitial space (Fig. 3).

A typical ECG, recorded in an oedematous animal is shown in Fig. 5. The top curves represent leads I and II recorded in the initial stage of anaesthesia. The next two curves show identical leads, 10 minutes after the injection of fluid and vasopressin. Heart rate somewhat diminished, otherwise there was no significant change in the shape of the curves. The lower recordings were made immediately before the animals was killed. Heart rate returned to previous values, the curves were essentially identical with those observed at the beginning of the experiment.

Discussion

It is generally believed that histological examination is the only infallible method of establishing the presence of pulmonary oedema. Some authors are of the view that pulmonary water content is not, but the ratio lung weight/body weight is an adequate indicator of pulmonary oedema (LUISADA and SARNOFF 1946). Other authors, however, do not accept these methods and suggest various tests for the purpose (GUYTON and LINDSAY 1959). In the present experiments the amount of fluid found in the lungs and the ratio pulmonary weight to body weight failed to show any consistent change in the animals with severe pulmonary oedema. This is in part consistent with the findings of other authors, nevertheless, the possibility cannot be excluded that the failure of pulmonary fluid content and of the ratio pulmonary weight to body weight to show a significant change might have been due to the circumstance that our method of inducing pulmonary oedema was entirely different from that used by others.

It has been known for long that the infusion of isotonic solution or plasma may lead to pulmonary oedema (WIGGERS 1939). The amount of fluid generally considered necessary for inducing severe pulmonary oedema is, however, 4 to 5 times as much as the amount of solution used by us. Moreover, there are some data indicating that even such immense amounts of fluid do not always suffice when given intravenously, and they cause oedema only when injected at high pressure into the carotid artery towards the brain (LUISADA and SARNOFF 1946). The observation that injection into the caudal vein of different solutions at a volume of 3 per cent of body weight did not induce pulmonary oedema was in agreement with expectations. Assuming that the fluid administered was evenly distributed in the extracellular space, one must count with an about 15 per cent decrease in colloid osmotic pressure, e.g. from 30 mm Hg to 26.5 mm Hg. This value is about twice as much as the level which is considered to be the threshold at which filtration of fluid into the alveoli usually starts (GUYTON and LINDSAY 1959). Nevertheless, this factor should be borne in mind, especially in the cases of cardiac failure when in the connective tissue similar amount of protein-free fluid might accumulate which, when reabsorbed during rest, increases circulating fluid volume.

Oedema failed to develop in 60 minutes if 100 mU of vasopressin, 50 mU intravenously and 50 mU intramuscularly, had been administered by itself. The authors studying the effect of vasopressin in nephrectomized rats have never observed pulmonary oedema from doses up to 500 ml (FRIEDMAN et al. 1956, HALTZ 1932). A dose of vasopressin similar to that injected intravenously in the present experiments causes an increase of 40 to 50 mm Hg in systemic blood pressure and of 10 to 15 mm Hg in pulmonary arterial pressure. Similar results were obtained by others in the rat (FRIEDMAN et al. 1956). The effect on blood pressure was studied by us in dogs (but not in rats) and it was found that the increase in blood pressure disappears in 10 to 15 minutes and a second intramuscular injection of 50 mU vasopressin failed to display any considerable effect. The rise in blood pressure was in all probability responsible for the transient bradycardia (Fig. 5). Whenever vasopressin had been given in association with fluid, severe pulmonary oedema developed in 60 minutes in almost every animal. There was practically no difference between the experimental groups, although hyperosmotic glucose is commonly used in clinical practice for controlling pulmonary oedema.

Another possibility is that acute left ventricular failure, leading to an increase of pulmonary venous pressure, was responsible for the pulmonary oedema. It has been demonstrated that vasopressin given intravenously elicits coronary spasm (PAPP and SZEKERES 1965); the consequential anoxia might then lead to cardiac failure. However, the ECG recordings showed no sign of cardiac anoxia, the fact that vasopressin by itself did not cause pulmonary oedema indicates that left ventricular failure could not be the only factor accounting for the oedema.

In the absence of other factors increased pulmonary arterial blood pressure does not suffice for pulmonary oedema to develop; it might even relieve the oedema by decreasing pressure in the arterioles. Supposing that increased blood pressure had still contributed to the development of pulmonary oedema, the filtration of fluid into the alveoli must have occurred in the first 10 minutes of observation, and in the remaining period of the experiment this fluid must have been reabsorbed and transported by the lymphatics. In our earlier experiments concerned with problems of renal function, where the administered amount of fluid and the dose of vasopressin were similar, the majority of animals died in 60 to 90 minutes. Thus, the present form of experimental pulmonary oedema seems to develop progressively and what we observed in the 60th minute was not the terminal phase, since the animals did not yet suffocate. This was the reason why we chose the 60th minute for killing the animals. It is highly improbable that filtration of oedema fluid had taken place during the first 10 minutes.

A further possibility is that vasopressin causes the pulmonary lymph vessels to constrict (FÖLDI et al. 1954). The only objection that might arise

against this assumption is the lack of contractible elements in the lymph vessels. The excessive dilatation of lymphatics in the histological sections is, however, a strong proof of there being such a mechanism. In all probability, the lymph vessels are insufficient for the transportation of increased amounts of ultrafiltrate (Figs 2, 3 and 4). Augmented ultrafiltration, on the other hand, seems to be due to the diminished osmotic pressure of plasma, and partly to the vasopressin. Although vasoconstriction brought about by vasopressin might inhibit the formation of oedema, there are nevertheless data indicating that the drug is increasing permeability (SCHOFFENIELS and TERCAFS 1962, HAYS and LEAF 1962, FONG et al. 1960). We are of the view that in the present experiments the main factor giving rise to oedema was the increased permeability of pulmonary capillaries; this allowed a considerable amount of fluid to be filtrated from the hyposmotic plasma. Obstruction of the ureters made it impossible for the animals to eliminate the administered fluid, hence the hyposmotic condition was maintained for a considerable length of time. Under such circumstances, the insufficiency of pulmonary lymphatics caused an accumulation of fluid in the alveoli. This phenomenon deserves some attention from the clinical point of view, too, as the applied dose of vasopressin was about twice the usual 25 to 27 I. U. dose for an adult weighing 60 kg. The question remains open as to which of the fractions of vasopressin was responsible for the development of pulmonary oedema, and to what extent other organs were involved in this process.

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MULTIPLE PARATHYROID ADENOMA

CLINICAL, HISTOLOGICAL AND ELECTRON MICROSCOPICAL STUDIES

By

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A case of concurrent double parathyroid adenoma and of the adrenal cortex has been reviewed. By close analysis of the clinical features and of histologic and ultramicroscopic finding of the removed tissue the hormone-producing capacity of the clear chief cells has been substantiated.

The concepts of tertiary hyperparathyroidism and of relative hypercalciuria have been discussed. The necessity for early surgery in cases suspect of parathyroid adenoma, regardless of the condition of the kidneys, has been stressed.

The debates over the origin of parathyroid adenoma are gradually subsiding since in the light of accumulating clinical evidence it becomes more and more accepted that continuous exposure of the glands to an irritation of some kind leads to their hyperplasia. The most common source of such irritative stimuli is hypocalcaemia which may be the consequence either of intestinal or of renal abnormalities or of corticosteroid treatment [17]. The stimulus of hypocalcaemia has been found to increase the production of parathormone *in vitro* as well [30, 31]. Disturbances of magnesium metabolism may likewise stimulate parathyroid function [15, 20, 21, 22, 23].

Inflammatory processes in the tissues adjacent to the parathyroid glands, particularly thyroiditis [26], furthermore permanent experimental acidosis [11] are further irritative factors which may be at the origin of parathyroid hyperplasia.

If the irritative stimulus responsible for hyperplasia becomes permanent, it may lead to an adenomatous transformation of the hyperplastic tissue.

On the grounds of these facts hyperparathyroidism, whether primary or secondary, may be regarded as one and the same entity, the more so as at later stages it becomes undistinct which of the two patterns has been originally present, since both involve eventually the kidneys which again adds to the excitatory stimulus affecting the gland.

These functional relationships allow a better understanding of the cases of multiple parathyroid adenoma or those of concurrent parathyroid hyperplasia and adenoma reported in the literature.

By the description of the present case it is intended to illustrate the above outlined mechanism and to contribute to the ultrastructural evidence.

History and findings

1. Clinical investigations

R. J., a 69-year-old male was admitted on 16.7. 1965. He had been experiencing pain in his left thigh and difficulty in moving his legs since 1952, without deriving any benefit from physical and drug treatment. In 1961, when he had been hospitalized for some other disease, the cause of his extremital pains was investigated and hyperparathyroidism was diagnosed, the serum calcium level having been between 12.4 and 16 mg per 100 ml. The increasing severity of the symptoms has made reinvestigation necessary in 1964. The findings including the radiographic evidence of a walnut-sized defect in the left tibia were consistent with hyperparathyroidism. In February, 1965, he had felt a sudden splitting pain in the left leg; this had made a further impairment in standing up and in walking. On renewed hospitalization the serum calcium was found to have attained 16 mg and the NPN, 57 mg per 100 ml. Since then he had been on a low-calcium diet and on a maintenance therapy with prednisolone with few interruptions. This had kept the calcium level between 11 and 14 mg per 100 ml.

On admission he complained of pains, constipation and polyuria. The conspicuous features were a considerable kyphosis, tenderness of the bones and a liver reaching two fingers below the costal arch. The laboratory findings were: serum calcium between 10 and 15.4 mg per 100 ml; inorganic phosphorus between 3.8 and 5.5 mg per 100 ml; citrates between 2.2 and 3.1 mg per 100 ml; urinary calcium excretion, 330 mg and after a calcium-free diet 405 mg in 24 hrs; pH of the urine was 6.4, it could not be further acidified. Tubular phosphorus reabsorption was 55 per cent repeatedly. GFR was 40 ml. There was a diffuse decalcification with subperiostal erosions while the thickness of the skull exceeded the average values. The QT in the ECG measured 0.28″.

The results clearly confirmed the existence of parathyroid adenoma. Progressive deterioration of renal functions as well as of the general condition called for surgery (Prof. I. LITTMANN). After the removal of a structure occupying the right upper pole of the thyroid gland (it proved to be fat tissue), a plum-sized mass was felt below the right lower pole of the thyroid gland, protruding retrosternally. After its removal, in the left thyroid lobe no tissue suggestive of adenoma could be detected. The first three postoperative days were uneventful. On the fourth day the patient became confused, restless and developed urinary retention. He was retransferred to our department where he became still more confused, NPN rose steadily while GFR fell to 15.5 ml. Intermittent incomplete urinary retention due to prostatic hypertrophy made the insertion of an indwelling catheter necessary. After fluid antibiotic and digitalis treatment the patient died on the 15th postoperative day. On the first postoperative day serum calcium was 10.8 mg per 100 ml, citrate 2 mg per

100 ml, and on the following days these values were 5 mg per 100 ml and 1.7 mg per 100 ml, respectively, calling for vitamin D and AT 10 treatment. The alkaline reserve was 29.1 vol. per 100 ml. The falling calcium levels were in contrast with the tubular phosphorus reabsorption of 75 per cent, thus closer to the hyperparathyroid pattern.

The removed tissue was submitted to light- and electron microscopic study.

2. Histological study

A plum-sized fawn-coloured tissue fragment of firm, glandular consistency, enclosed by a membranous capsule was submitted to histological examination.

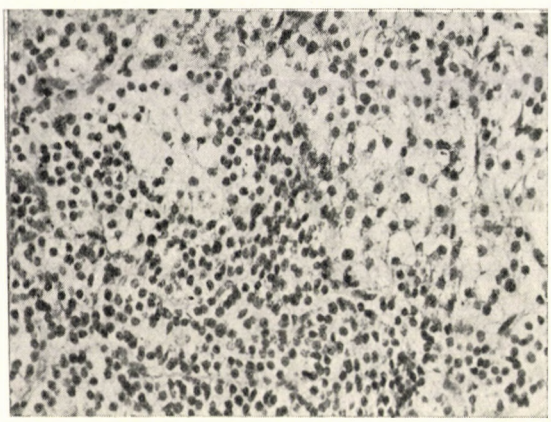

Fig. 1. Light microscopic view of the chief cells and water clear cells — intermediate and pure types — of parathyroid adenoma. The chief cells are characterized by an eosinophile narrow cytoplasmic rim and a rotund granulated nucleus. The water clear cells of both intermediate and pure types have a broader cytoplasm than the chief cells, and a foamy, vacuolated pattern; some of them appear optically empty

The sections revealed parathyroid tissue broken up into islets by fibrous bundles of various thickness, and made up in focal arrangement of different cell types.

The first cell type (Fig. 1) showed on HE-staining a relatively narrow pale cytoplasm with eosinophile granules and centrally situated ovoid nuclei. The nuclear chromatin was coarsely granulated. The cells contained one or two distinct nucleoli.

The other cell type (Fig. 1) was characterized by a broader, foamy, vacuolated, often water clear cytoplasm and a dense, eccentric, even marginal nucleus with distinct nucleoli. The former type corresponded to the chief cells, the latter to an intermediate form of the water clear cells of the parathyroid gland. Typical water clear cells also occurred, either individually or in focal

arrangement. There was, moreover, a wide variety of intermediate types, particularly at the periphery of the foci made up of the individual cell types, but occasionally entire foci consisted of intermediate cells. In the present case, however, the tumour was largely made up of the two main cell types arranged into fields of various sizes.

There were also occasional smaller islets of a third cell type (Fig. 2). These cells were slightly larger than those of the other types, sharply outlined,

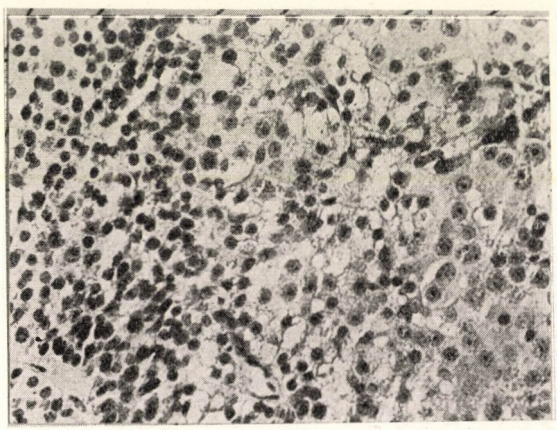

Fig. 2. Chief cells and oxyphile cells from human parathyroid adenoma. The oxyphile cells are larger than the chief cells, their cytoplasm is markedly eosinophilic. The nuclei generally occupy the marginal area of the cell, they are rotund or ovoid with a coarsely granulated chromatin structure

and showed a broad, definitely acidophile cytoplasm. The ovoid (or rotund) nuclei containing coarse granulated chromatin were usually marginal. These cells resembled normal parathyroid cells.

The cellular islets were interspersed with cross-sections of blood and lymph capillaries with a single endothelial layer as their lining. The fibrous stroma showed in some areas hyaline transformation with pigmented macrophages. At sites, circumscribed haemorrhages or definite fibrotic changes were seen. In contrast to the normal parathyroid pattern, the sections under study showed next to no fat tissue.

In respect to its microscopic features the adenoma thus belonged to the mixed type.

3. Electron microscopic study

Electron microscopy showed a prevalence of light chief cells (Fig. 3). These were polygonal and tended to form islets of various sizes. They were tightly packed together, their membranes being strongly creased, occasional desmosomes providing for a tight adherence were present. The nuclei were

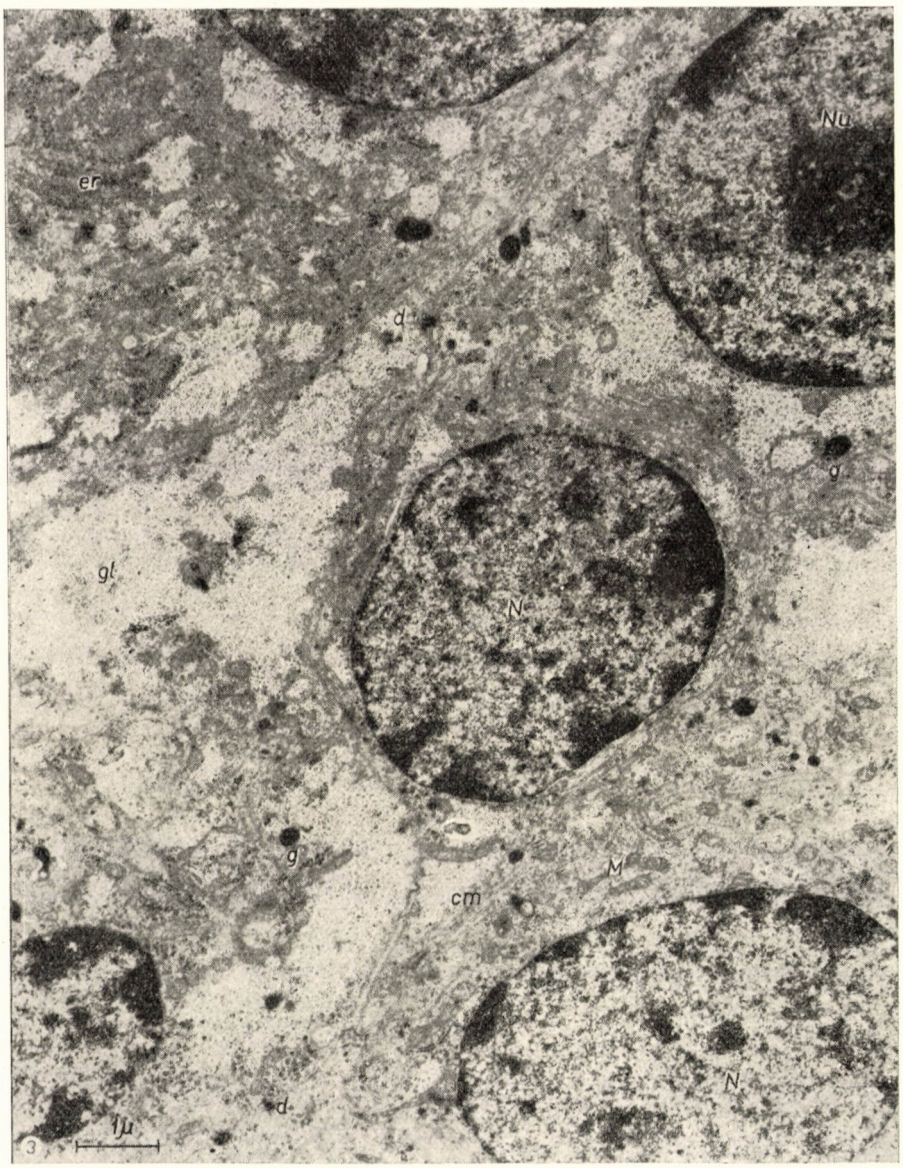

Fig. 3. Light chief cells of human parathyroid adenoma. The nuclei (N) are rotund, with a moderately electron-dense chromatin structure. The nucleolus (Nu) usually occupies the nuclear centre. The cellular membrane shows marked wrinkling, often with desmosomes (d). The conspicuously clear appearance of the cells is due to extensive intracellular glycogen fields (gl) of strong electron-permeability. The cytoplasm contains, in addition to glycogen, endoplasmic reticulum (er) in considerable amounts and mitochondria (M) as well as secretory granules (g) in variable numbers. × 15 300

round or ovoid, with unevenly distributed chromatin tending to cluster along the nuclear membrane. The nucleolus which mostly occupied the centre of the karyoplasm, consisted of a strongly electron-dense and a moderately electron-dense, finely granulated moiety.

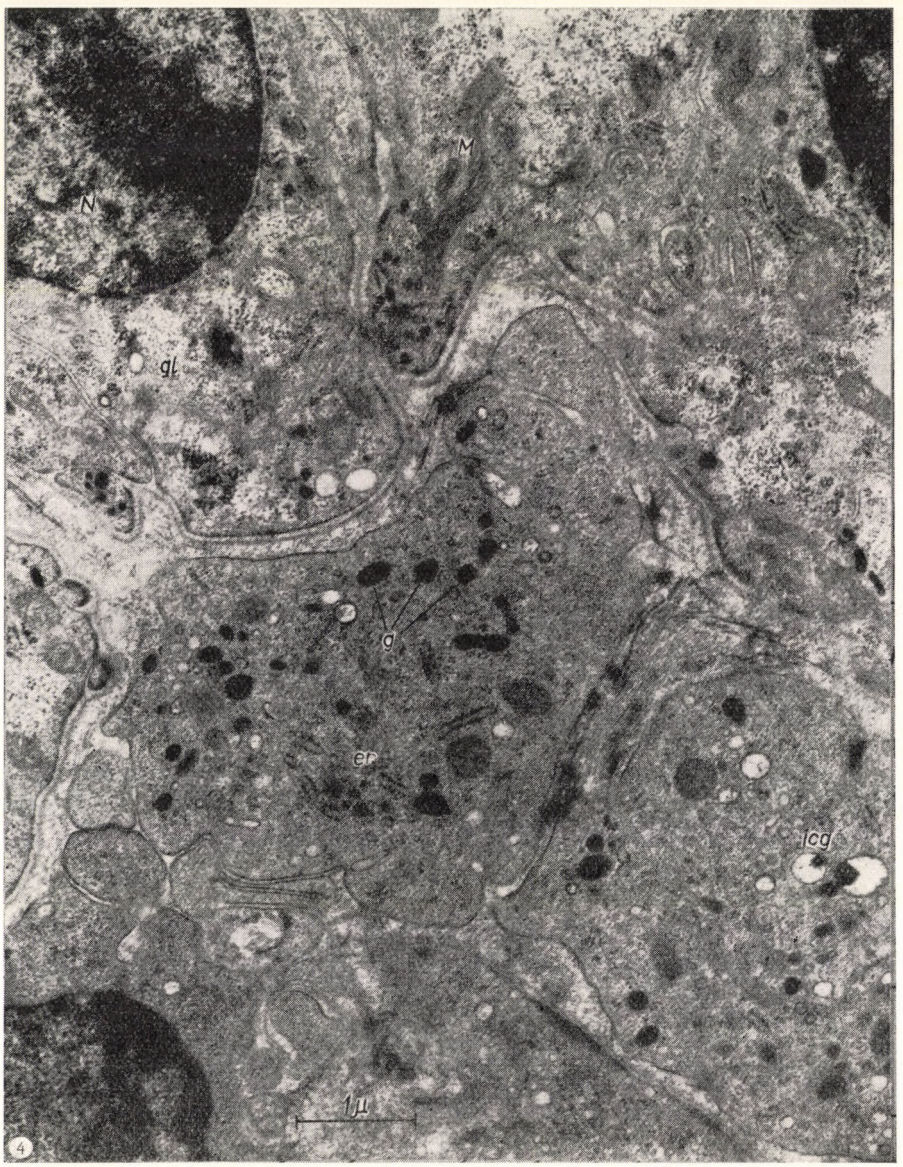

Fig. 4. Dark chief cells of human parathyroid adenoma characterized by a cytoplasm of high electron-density with numerous secretory granules (g) of diverse shapes and sizes (0.1—0.5 μ) and of occasional intracisternal localization (icg). The cytoplasm furthermore contains a fully developed endoplasmic reticulum (er), mitochondria (M) of varying number in each cell and glycogen granules (gl). × 23 000

The chief cells resembling the clear type were characterized by a clear cytoplasm of poor electron density, excessively rich in glycogen granules, but poor in lipid droplets, mitochondria and secretory granules. The glycogen granules of irregular star-like shape varied in size (200 to 400 Å) and density. There was a varying amount of coarse endoplasmic reticulum displaying a lamellar pattern.

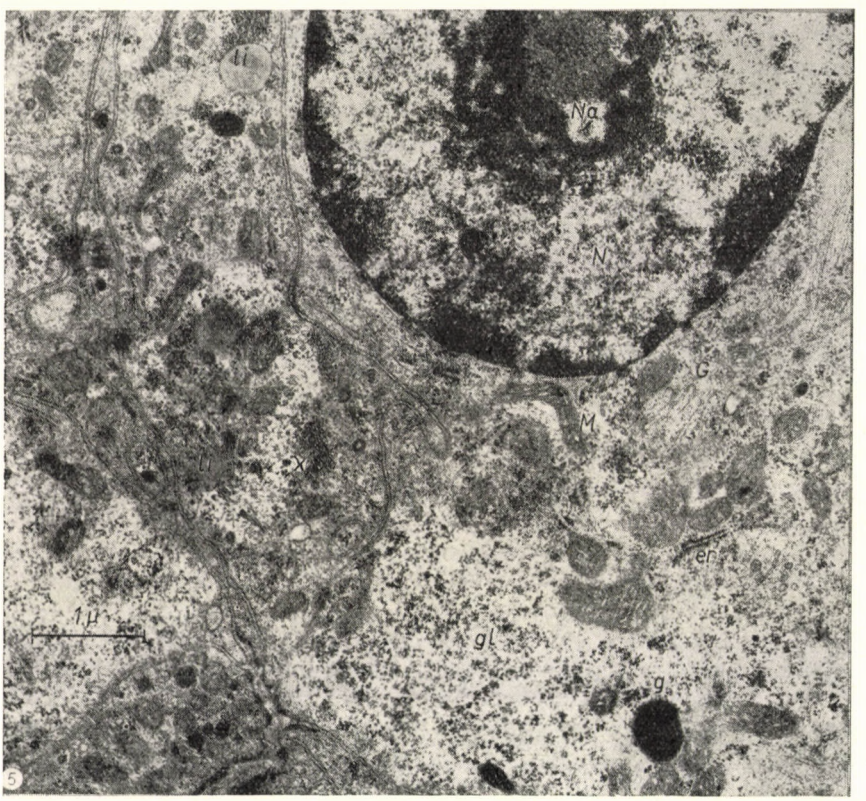

Fig. 5. Intermediate chief cells whose cytoplasm combines the features of the light and the dark cells. Despite its richness in glycogen (gl) the cytoplasm is only moderately electron-dense. It contains numerous mitochondria (M), lipid droplets (li), and secretory granules (g). The Golgi apparatus (G) is poorly developed, the endoplasmic reticulum (er) is likewise scarce. In some of the cells granular conglomerates of high electron-density (x) may be observed × 22 400

The dark chief cells (Fig. 4) making up the minor part of the adenoma contained a strongly electron-dense dark cytoplasm, a rich, lamellar endoplasmic reticulum, a variable number of mitochondria and numerous secretory granules. Accordingly, they were considerably poorer in glycogen than the light chief cells. They were more loosely arranged than the former, and the cytoplasm often showed sharply outlined processes protruding into the wide

intercellular space. They were poorer in lipid droplets, too, but contained numerous empty vesicles and mitochondria, as well as Golgi apparatuses of vesicular appearance, occasionally ciliar cross-sections. Some of the cells constituting an intermediate form of the two types (Fig. 5/x) contained conspicuous conglomerates of a strongly electron-dense substance of filamentous arrangement.

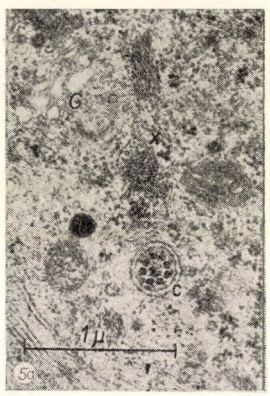

Fig. 5a. Cross-section of "cilium" of atypical 8 + 2 arrangement, bordered by granular conglomerates (x) and part of a Golgi apparatus (G) in a detail of a parathyroid adenoma cell

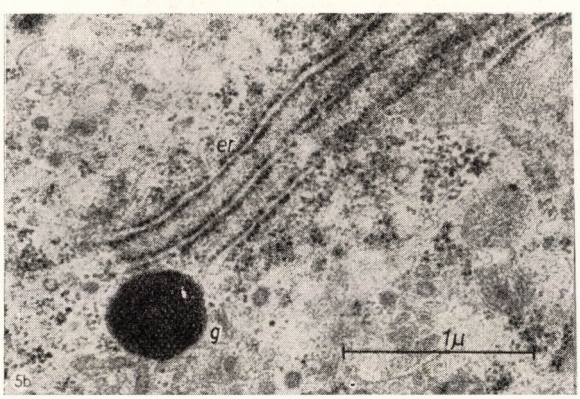

Fig. 5b. High-power view of a secretory granule (g). Its microgranular structure and its close morphologic relationship with the endoplasmic reticulum appear clearly

The conglomerates have not been identified chemically, on the other hand, they were clearly discernible from glycogen or ribosome granules.

The secretory granules were rotund, ovoid or rod-shaped. Some of them were located intracisternally. This was of interest since no such arrangement of the secretory substance has been described in normal or in adenomatous parathyroid cells, therefore the said location may well represent a special pattern of condensation and elimination of the secretory material.

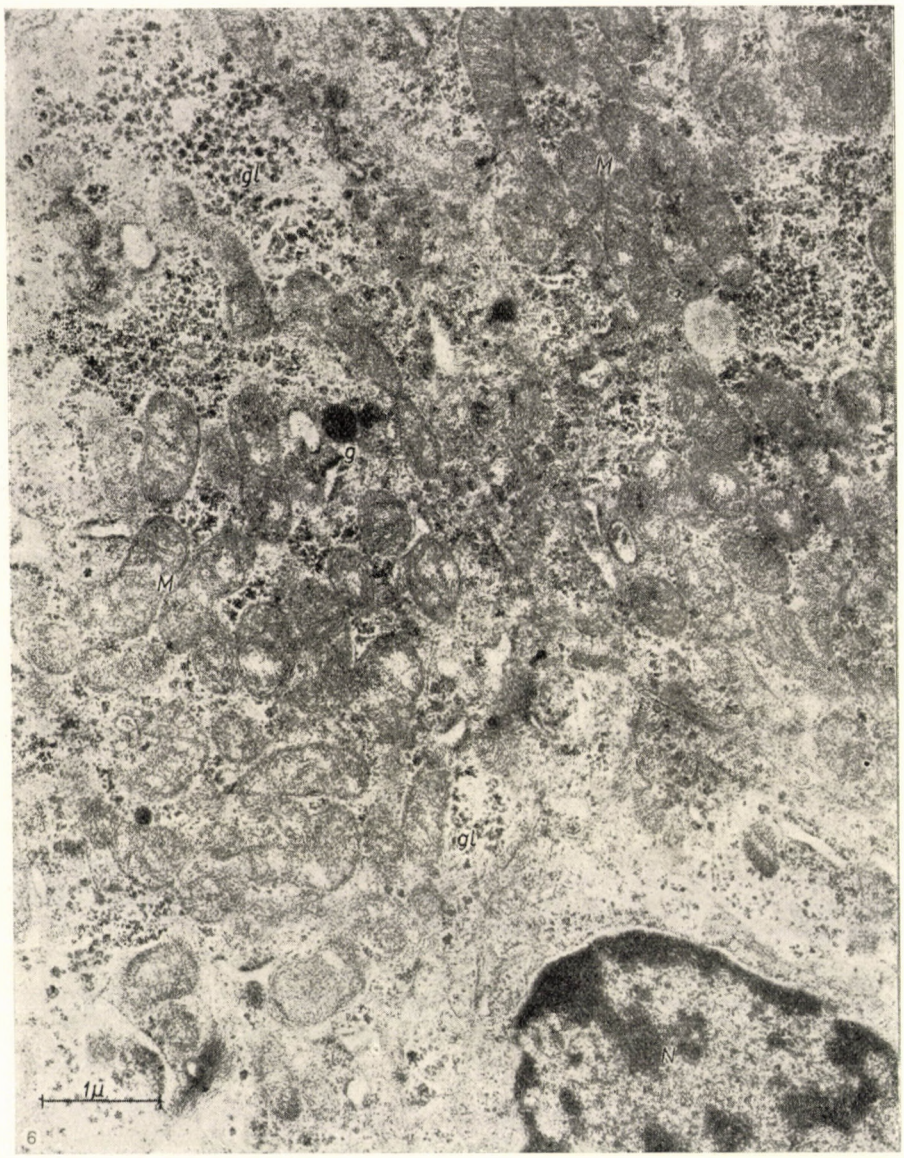

Fig. 6. In the oxyphile cells of the adenoma the cytoplasm is almost completely filled with closely packed mitochondria (M) and with glycogen granules (gl) in their vicinity. Sparse secretory granules (g) may also be seen. × 24 000

The oxyphile cells (Fig. 6) had a larger cytoplasm than the chief cells with numerous, tightly packed rod-shaped mitochondria characterized by typical cristae, and a moderately electron-dense and occasionally vacuolated matrix. Lipid droplets and secretory granules were scarce while the glycogen

occupied a more or less large area of the cytoplasm. Golgi apparatuses and endoplasmic reticulum were scarce.

Water clear cells were observed only occasionally.

4. Post-mortem findings

Two of the remaining parathyroids, i.e. the left and the right upper glands were identifiable, the former pea-sized, the latter bean-sized. The structure which had been believed to be the left lower parathyroid, subsequently proved to be thyroid tissue. The left upper parathyroid revealed a normal microscopic pattern while in the right upper gland there was a hyperplasic or adenomatous focus largely made up of proliferating clear cells; it was sharply demarcated from a surrounding narrow rim of parathyroid tissue formed mainly of chief cells. Another interesting finding was an enlargement of the adrenals, the left weighing 20 g, the right 15 g; the cut surface showed a sharp demarcation between cortex and medulla. In both glands small bean-to-plum-sized bright yellow cortical adenomas were found. The pituitary gland was normal.

The two kidneys weighed 180 g. Under the easily detachable fibrous capsule a greyish red, granular surface appeared showing, among miliary prominent foci alternating with retracted areas, thin-walled vesicles filled with fluid. Occasional pea-sized, poorly outlined abscesses were also seen. On the cut surface the cortex appeared narrow, poorly demarcated from the medulla. The pyramidal area showed a yellowish white streaking. The calices were dilated. The lower calices of both kidneys contained each an irregular pea-sized calculus. The renal pelves were moderately dilated, filled with turbid fluid, the mucosa was swollen, congested, finely vascularized. The renal findings were thus indicative of chronic apostematous pyelonephritis, bilateral nephrolithiasis and nephrocalcinosis.

Discussion

Parathyroid adenoma usually appears as a solitary node confined to a single gland [14, 28, 29], prevalently in one of the lower glands, but it may arise from an aberrant parathyroid as well. Reports on multiple adenomas are scarce [3, 9, 5, 16, 32].

Since the first detailed study of parathyroid abnormalities by CASTELMAN and MALLORY [9] as far back as 1935, reports on this subject have been scanty [6, 39]. The study of WOOLNER et al. [39] deserves special attention.

These authors divided the solitary parathyroid adenomas into five types. There is, however, much disagreement about the incidence of the individual types [3, 5, 32, 39]. Though from the histogenetic aspect, the morphological type of the adenoma certainly has its significance, from the clinical point

of view it matters very little to which type it belongs, since no definite correlations have been found between the cellular pattern of the tumour and its functional properties.

In differentiating parathyroid adenoma from hyperplasia it is helpful to remember that hyperplasia involves all parathyroid glands, since the cell type alone will hardly ever decide the diagnosis, despite certain clues, thus for instance in primary hyperplasia practically 100 per cent of the cell population belong to the water clear type while in secondary hyperplasia the chief cells of normal size are prevalent. In adenomas any of the described types may predominate or mix as seen in the present instance. Electron microscopy is still less suited for the differentiation of adenoma from hyperplasia. The promise of this method lies in a closer identification of the cell type through its ultrastructure, thus allowing to gain a better insight into its functional activity than evidence revealed by light microscopy.

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Apart from electron microscopic investigations concerning the normal parathyroid [19, 25, 26, 27, 34, 37] the abnormal ultrastructural changes of the gland have also been studied [35, 36, 36a]. This work revealed that in adenoma as well as in primary chief-cell hyperplasia of the parathyroid, the same cellular types may be identified as in the healthy gland. According to ROTH [35] the most common type of parathyroid adenoma is made up of light chief cells. In our own electron microscopic material this cell type was likewise prevalent. Certain ultrastructural features of these cells, namely the presence of large amounts of coarse endoplasmic reticulum, of mitochondria and a variable number of secretory granules, apart from the glycogen granules, are associated with active hormone production. The claim that the clear chief cells are inactive [36] is invalidated by the electron microscopic findings as well as by the present case where an adenoma made up exclusively of such cells, was associated with clinical hyperactivity. ROTH [36] too alleges that secretory hyperactivity of the gland is associated with scanty secretory granules. The clear appearance of the cells is due to their high glycogen content and to the paucity of secretory granules, whereas the massive amounts of intracellular glycogen have been connected by ROTH with the special function of the adenoma cells which he believes to be independent of the neuro-hormonal regulatory mechanisms of the organism. In our opinion, the clear cytoplasm of the cells is by no means incompatible with an endocrine function. It means on the contrary that mobilization of the hormone produced in abundance by the hyperactive adenoma cells is too fast to permit an intracellular deposition of secretory granules. On the other hand, the dark chief cells which are excessively rich in secretory granules and endoplasmic reticulum and appear to belong to the storing type of chief cells may be found in considerably greater numbers

in the normal gland than in adenomas [27]. In agreement with the findings of light microscopy, the mixed cell adenomas yield the electron microscopic evidence of oxyphile cells in which secretory granules are scanty.

As an explanation of the rarity of adenoma cells with Golgi apparatuses it may be assumed that the production of parathormone falls to the rich ergastoplasmic structure, a possibility not only suggested by the abundance of ergastoplasm but also by its close morphologic relationships with the secretory granules.

*

The present case had various points of interest.

1. The incidence of multiple parathyroid adenoma is very low; in fact, not more than 4 per cent of parathyroid adenomas are multiple. The only observation of its occurrence together with adenomas of other endocrine organs has been reported by GOLDEN [1]. WERNER [38] claims causal relationships between the origin of multiple hormone-producing adenomas and autosomal gene injury.

In opposition to ZOLLINGER—ELLISON's syndrome, they invariably produce organ-specific hormones instead of gastrin.

2. Electron microscopic studies have provided contributive evidence to the question of functional activity by demonstrating that secretory granules carrying the polypeptide hormone may appear intracisternally, furthermore, that the chief cells abounding in glycogen, are hormone-producing.

3. The third aspect of the present study is that of "tertiary hyperparathyroidism" which in the process of primary hyperparathyroidism arises as a consequence of the renal complication, i.e. of nephrocalcinosis or pyelonephritis, and calls for surgery [2, 7, 12, 13, 24] in the same way as does parathyrotoxicosis [4].

Hypercalcaemia and hypercalciuria are known to cease gradually, parallel with the progress of secondary hyperparathyroidism. Accordingly, normal calciuria associated with a low GFR may signify a "relative hypercalciuria" [13]. In opposition to other renal diseases, calciuria of this origin is not reduced by a calcium-free diet. In secondary hyperparathyroidism of "regulatory" character, hypocalcaemia and hypocalciuria due to malabsorption are further impaired by a calcium-free diet. The serum citrate is high in both types, while in "regulative" hyperparathyroidism, calciuria increases as a response to calcium infusion [26a].

Several instances are known in which hyperplasia of an endocrine organ subject to excitatory stimuli results in neoplasm [13]. WOOLNER [39] asserts on the other hand that there are no sharp limits between true neoplasm and nodular hyperplasia of an endocrine organ. Nodular hyperplasia may represent the first stage.

The present case illustrates once more that removal of one adenoma is not always sufficient for stopping the hyperparathyroidism. Careful exploration of all four glands at surgery is of paramount importance. Successful operation for adenoma must be checked at a later time by pelvic bone biopsy [1], as well as by the necessary biochemical and tolerance studies.

There was no possibility of establishing in retrospect whether the bilateral adrenocortical adenomas represented an incidental finding or whether hypercorticoidism had actually been the primary change giving rise to hyperparathyroidism through a transitory hypocalcaemia [17], since the results of adrenocortical function studies dating from the early stages of the disease were not available.

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BLOOD GASES IN POLYCYTHAEMIA VERA

By

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Blood gas analyses have been performed in 37 patients with polycythaemia vera on 82 occasions. The abnormal findings demonstrable during exacerbation are ascribed to the high erythrocyte count and circulating blood volume.

Polyglobulia may signify a primary disease, as in the case of the entity of unknown aetiology, generally known as polycythaemia vera, or else a secondary condition. Secondary polyglobulia comprises two types. The first results from an oxygenation deficit in pulmonary or cardiac diseases associated with functional insufficiency. In these cases the increase in circulating blood volume and in the number of erythrocytes results from a compensatory mechanism. The other type may be connected with endocrine dysfunctions (e.g. Cushing's disease or syndrome), or with tumours of various systems, particularly of the kidneys [3, 17, 20], the liver [4, 8, 11], the chromaffine system [1, 5], and intracranial tumours [21].

The rise in blood volume and red cell number, as well as the hypercellular bone marrow and the short life-span of erythrocytes [20] associated with polycythaemia vera are signs of an increased haemopoiesis.

The interest of blood gas studies in polycythaemia vera lies not only in the possibility of obtaining characteristic values which might help to differentiate this condition from secondary polycythaemia [12]. It is also expected that by a comprehensive evaluation of the results of gas analysis and of parallel erythrocyte counts, correlations could be found between the values for blood volume and viscosity and the severity of oxygenation defect.

Material and methods

We have been systematically studying and following up patients with polycythaemia vera since 1959. The respective therapeutic results have been reported earlier [6, 7, 15]. The present study reports on the results of blood gas analysis performed in a number of cases and compared to the haematologic status.

A total of 37 patients was studied, 24 of them at various intervals. In addition to O_2 saturation and pH of arterial blood which was determined in all of the cases, arterial pCO_2 and pO_2 were studied in 26 patients. Differential blood counts, bone marrow smears, haematocrit, haemoglobin value as well as the clinical state were checked at regular intervals and it was on these grounds that the patients were considered to be in relapse or in remission. A red

count above 5 million, haemoglobin above 17 g per 100 ml, a hypercellular bone marrow, hepato-splenomegaly, typical symptoms, such as headache, itching, haemorrhagic diathesis were regarded as signs of exacerbation. The total number of blood gas studies performed in 37 patients was 82. 72 of these tests were made during exacerbations, 10 during remissions.

Of the patients 12 were females, 25 males. Their age ranged between 27 and 69 years, the majority were between 40 and 60 years of age. Congestive heart failure of sclerotic, pulmonary or valvular origin, present in 11 cases, could be kept under control by digitalis treatment. Eight patients had hypertension responding well to drug treatment.

The blood gas technique has been described earlier [18].

Results

The results fall into two groups according to the patients being in relapse or in remission at the time of study:

During remission, 10 determinations of arterial O_2 saturation, 10 of arterial pH, 2 of pCO_2, 2 of pO_2; during exacerbation, 72 determinations of arterial O_2 saturation, 72 of arterial pH, 29 of pCO_2, and 29 of pO_2 have been performed.

The results together with the number of measurements, the mean values for the individual groups and the standard errors are presented in Tables I and II.

Table I

Blood gases during exacerbation

Parameter	Number of measurements	Mean value	Standard error
Arterial O_2 saturation, per cent	72	93.8	$\pm$0.80
Arterial pH	72	7.4	$\pm$0.405
Arterial pCO_2, mm Hg	29	43	$\pm$15.20
Arterial pO_2, mm Hg	29	82.5	$\pm$15.10

Table I shows that exacerbation was accompanied by a moderate reduction of arterial O_2 saturation, a definite reduction of pO_2, normal arterial pH, and an increased pCO_2.

Table II

Blood gases during remission

Parameter	Number of measurements	Mean	Standard error
Arterial O_2 saturation	10	95.8%	$\pm$0.45
Arterial pH	10	7.39	$\pm$0.68

During remission, O_2 saturation and pH were at normal range, pO_2 was 94 mm Hg in one and 95 mm Hg in another patient, while pCO_2 was 40 mm Hg in both patients.

The differences between the two groups gain in significance by the fact that the values obtained during remission were invariably derived after ^{32}P treatment, of patients whose values determined prior to this treatment figure in the exacerbation group.

Discussion

The high erythrocyte count and circulating blood volume associated with many cases of secondary polyglobulism is due to a compensatory mechanism arising as a consequence of hypoxia, as in the case of congenital valvular defects or of chronic cor pulmonale. It seemed interesting to study how the said changes affected the blood gas values. TIEDE and CHIEVITZ [19] failed to detect any significant change in the blood pH of patients with polycythaemia vera. LOTZMAN et al. [10] found an arterial O_2 saturation of less than 80 per cent in 11, and less than 92 per cent in 9 out of 26 patients with polycythaemia vera; they ascribed the decrease to the increased blood volume and red cell count. BURGESS and BISHOP [2] studied the diffusion capacity in the stages of exacerbation and remission of polycythaemia vera. The values were found to be reduced during exacerbation and normal during remission.

NEWMAN and FELTMAN [16] observed a decreased O_2 saturation and increased pCO_2 in polycythaemia vera. By the application of current methods, two members of our team [18] found blood gas abnormalities in cardio-respiratory failure; it has been concluded that the elevation of haemoglobin level, in other words, secondary polyglobulia which provides compensation for hypoxaemia at first, fails to do so at later stages of functional impairment when cardio-respiratory failure has become manifest.

On the evidence of the present studies, exacerbations of polycythaemia vera are accompanied by a moderate decrease of O_2 saturation, a definite reduction of pO_2 and an increase of arterial pCO_2. Abnormal gas exchange reveals itself in the first line by the changes in the pO_2 and pCO_2 values. (Incidentally, owing to technical reasons, we had to confine the determinations of pO_2 and pCO_2 in remission to two cases, and to correlate the values obtained during exacerbation to those generally known from literature.) On these grounds, the abnormal values may be viewed as being due to the increase in the number of red cells and in the volume of circulating blood.

Exacerbation of polycythaemia vera and secondary polyglobulia are associated with deficient oxygenation. The distinctive point is, however, that while in polycythaemia vera these values are normalized parallel with the response to treatment of the erythrocyte count and the haemoglobin level, in

secondary polyglobulia the reduction of the erythrocyte count and of the haemoglobin level has an adverse effect on oxygenation. It follows that the results of blood gas analysis must be viewed in the light of their response to adequate treatment in order to be of any use in the differentiation of polycythaemia. One of the present authors [14, 15] noted haemorrhagic disorders in cases of polycythaemia vera during exacerbation. Parallel with the normalization of the platelet count, these changes, too, were found to respond to therapy. All this, together with the evidence presented in the foregoing, would suggest that an increase in any type of cell population in the blood might alone lead to a functional impairment.

Results of respiratory tests will be reported in another paper.

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HAEMODYNAMIC CHANGES IN SHOCK ASSOCIATED WITH EXPERIMENTAL MYOCARDIAL INFARCTION

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Shock was seen to develop in 15 out of 22 animals subjected to experimental myocardial infarction.

1. Stroke volume and cardiac output fell in 19 instances; 12 of these were associated with shock.

2. TPR increased to double its initial value in 7 cases. In these, shock failed to develop. In 8 out of 12 experiments involving shock the TPR showed a moderate rise only and in 4, either none at all or even a drop. 3 animals developed shock despite an unchanged cardiac output. In these cases there was a considerable fall in TPR.

3. 5 experiments were designed to study cardiac output and TPR 30 and 60 minutes after coronary obstruction. Shock associated with a considerable fall of cardiac output as well as of TPR occurred in one instance only. In the other four cases, 30 minutes after coronary obstruction a reduction in cardiac output was registered but at 60 minutes the values had returned to normal.

4. A linear correlation was found between a) the coronary fraction of cardiac output and TPR; b) the renal fraction of cardiac output and TPR; and c) the coronary and renal fractions of cardiac output.

5. No correlation was found between coronary or renal flow and the other circulatory parameters.

Successful prevention and management of cardiogenic shock is greatly hindered by our ignorance of the underlying pathomechanism. Despite the wealth of clinical observations relating to myocardial infarction, the methods of studying this particular issue are not delicate enough to be really informative.

Attempts to induce shock in the laboratory animal by ligation of the coronaries or by electric or chemical damage to the myocardium have failed [1, 2]. AGRESS et al. have worked out a closed-chest method by which it is possible to induce myocardial necrosis followed in most cases by cardiogenic shock in dogs. Apart from slight modifications, it is the same method which we have been using in recent years for the production of myocardial infarction and cardiogenic shock in dogs, allowing to study the ensuing changes in stroke volume, cardiac output, blood pressure, calculated peripheral resistance, coronary and renal blood flow. The present paper will report on such studies.

Method

The experiments were carried out in 41 dogs of both sexes, weighing between 12 and 21 kg, under chloralose anaesthesia. Myocardial infarction was induced in 27 dogs by the modified method of AGRESS and BINDER [3]. Through a catheter inserted under radiographic control

via the femoral artery into the ascending aorta up to the level of the coronary orifices, 10 ml of a suspension containing agar globules of 300 to 350 μ size were injected. Previously a rubber balloon inserted through the left carotid into the ascending aorta was distended by means of 4 to 5 ml of acetrizoate in order to seal the aortic lumen for 15 sec, so as to prevent a dispersing of the globules to the periphery. Hermetic sealing was ascertained by microscopic examination of the liver, in some cases of the kidney. Cardiac output and stroke volume were determined by the dye dilution method [4] immediately before and 5 minutes after coronary obstruction in Group I, and also after 30 and 60 minutes in Group II. Peripheral resistance was calculated from the ratio of blood pressure and cardiac output. At the end of the experiment, i. e. 60 minutes after the intervention, the cardiac and renal fractions of cardiac output were determined by SAPIRSTEIN's method [5] and cardiac and right renal blood flow were calculated on the basis of that value. By correlating these figures with those of blood pressure found at the end of the experiment we estimated the vascular resistance of the heart and of one kidney.

As a control, in 14 healthy dogs cardiac and renal fractions of cardiac output were determined by means of ^{86}RS, without any other intervention. Right atrial pressure was estimated by heart catheterization.

Cardiac output (MV) was expressed in l/min; stroke volume (SV), in ml; total peripheral resistance (TPR), in cm dyne sec^{-5}; blood pressure (BP), in mm Hg; the cardiac fraction multiplied by per cent (Cfr), in ml/minute; coronary resistance (CR), in cm dyne sec^{-5}.

Results

Group I. In 7 out of 22 animals no shock developed (Table I and Fig. 1, group a). Blood pressure either remained unchanged, or its fall did not exceed 16 to 18 per cent of the initial value. Cardiac output was reduced to 36 to 66 per cent (mean, 44 per cent), parallel with an increase of TPR (mean, 92.7 per cent), while right atrial pressure remained unchanged. It may be inferred from the significant rise in TPR that the peripheral vasoconstriction arising as a mechanism compensating the reduction of cardiac output must have been significant enough to account for the absence of a fall in blood pressure.

Shock developed in 15 animals (Fig. 1, groups b, c, d). A reduction of cardiac output (by 40 to 70 per cent) was found in 12 cases. In 8 out of these, TPR showed a moderate rise, and in 4 it was unchanged or slightly diminished. Central venous pressure was unchanged. The fall of blood pressure was therefore due to the reduction of cardiac output. TPR showed that either no vasoconstriction had occurred at the periphery or even if it had, it was too slight for the compensation of the decrease in blood pressure accompanying the reduction of cardiac output.

In 3 cases (Fig. 1, d), blood pressure fell to 50 per cent of its initial level, but the reduction of cardiac output did not exceed 15 to 20 per cent. Right atrial pressure rose insignificantly. In contrast, TPR fell to 50 per cent of its initial value, pointing to peripheral vasodilatation.

In *Group II* the parameters were determined in 5 cases 30 and 60 minutes after coronary occlusion (Table II and Fig. 2). Cardiac output diminished successively in all of the cases; at 60 minutes it was around 50 per cent of the value determined at 30 minutes. Nevertheless, shock with a significant fall of blood pressure was found in one case only while in the other 4 cases the fall of blood pressure was confined to the first 30 minutes and did not exceed 10 to

Table I

	BP	CO	SV	TPR	Cfr	Rfr	CBF	RBF
1.	−17	−35	−57	+ 21	—	—	—	—
2.	−20	−35	−44	+ 20	—	—	—	—
3.	0	−55	−57	+123	7	21	99.5	298
4.	−16	−39	−62	+112	4.3	11.3	91.3	239
5.	0	−66	−69	+196	9.4	8.9	95	90
6.	−18	−36	−41	+ 47	6	26.5	109.9	485
7.	0	−42	−39	+ 72	7	22.5	67.2	216
8.	−33	−60	−52	+ 69	5.9	23.2	46.1	181
9.	−33	−69	−62	+115	7.4	23.6	102.2	326
10.	−38	−68	−72	+ 93	6	9.4	37.3	38.4
11.	−39	−51	—	+ 24	—	—	—	—
12.	−41	−52	−43	+ 22	4.2	23.4	46.6	260
13.	−47	−73	−74	+ 92	7.4	20.4	73.5	202
14.	−49	−54	−50	+ 14	4.6	14.0	25.3	77
15.	−50	−56	−48	+ 13	7.2	23.9	74.8	248.4
16.	−38	−30	−36	− 10	2	10.0	26	130
17.	−47	−46	−59	− 7	5.4	13.2	110.2	269.5
18.	−53	−46	−50	− 20	5.9	15.4	101.6	265
19.	−56	−51	−32	− 11	4.2	8.8	23.2	449
20.	−55	−17	+10	− 46	7.8	13.0	162.2	270.1
21.	−58	−21	−16	− 46	2.4	4.2	34.6	59.6
22.	−60	−16	−10	− 52	3.3	7.2	35.6	77.7

BP = blood pressure
CO = cardiac output
SV = stroke volume
TPR = total peripheral resistance
Cfr = coronary fraction of cardiac output
Rfr = renal fraction of cardiac output
CBF = coronary blood flow
RBF = renal blood flow

The figures represent percentual changes of the initial values

20 per cent. (In one of these cases, blood pressure was somewhat lower in the second 30 minutes, but in the other three cases, the 60 minute readings showed a return to the initial values.)

In the only case with shock blood pressure at 30 minutes was 33 per cent, at 60 minutes 58 per cent below the initial level. Reduction of cardiac output amounted to 29 per cent and 36 per cent, respectively, thus to not more than in the 4 cases with unchanged blood pressure. On the other hand, in these very four cases TPR successively rose in opposition to its significant drop in the only instance associated with shock (Fig. 2).

One of the objects of the present study was to find correlations between the individual parameters of circulation and the coronary fraction of cardiac

output, i. e. coronary flow. In 9 cases coronary flow increased, and in 6 it diminished in proportion to the respective change of TPR. It was in one case only that the TPR dropped by 45 per cent while the coronary fraction rose to 7.6 per cent. On the other hand, in 3 cases, despite a 15 to 20 per cent increase of TPR, the coronary fraction dropped to 4.2 to 4.6 per cent as against the normal 5.4 per cent. These exceptions were, however, too close to the limits of the normal

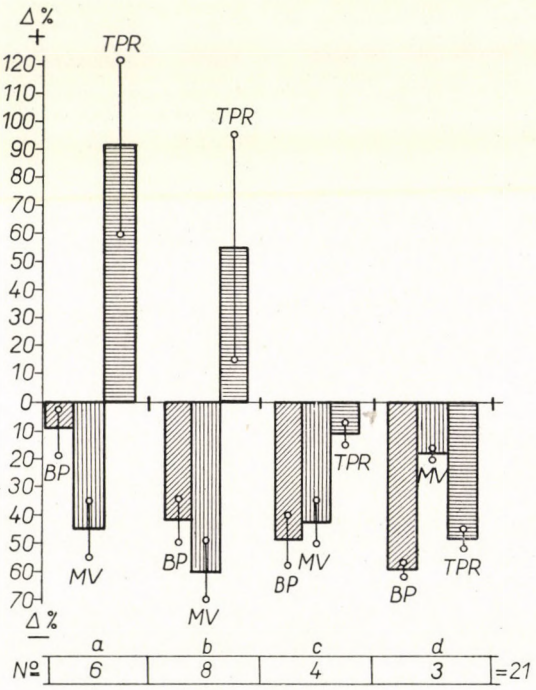

Fig. 1

range to be necessarily pathological (Fig. 3). Similar correlations have been found between the changes of TPR and the renal fraction of cardiac output (Fig. 4). With the exception of 3 cases the renal fraction increased or diminished in proportion to the TPR.

The existence of a correlation between TPR and the coronary and renal fractions of cardiac output has thus been demonstrated by the present experiments.

The values for cardiac output and its organ fractions allow to define blood flow in a particular organ. Table I shows the changes of coronary and renal flow in shock. It is seen that, with the exception of two cases, coronary flow diminished throughout regardless of the behaviour of the coronary fraction of cardiac output. Coronary flow showed no correlations with blood pres-

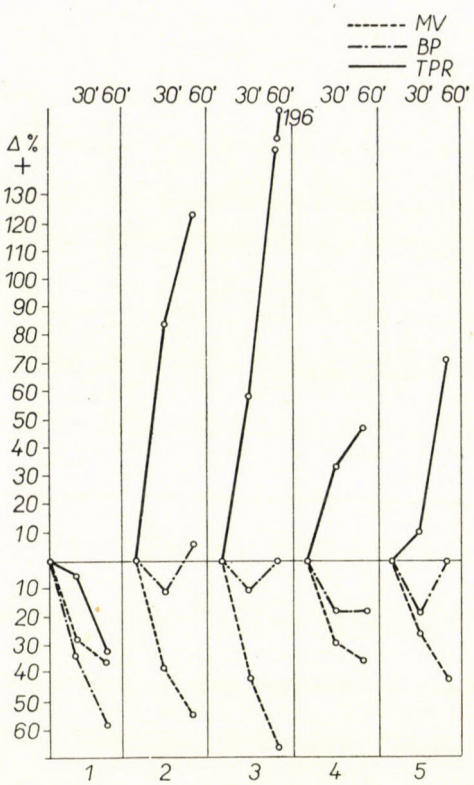

Fig. 2

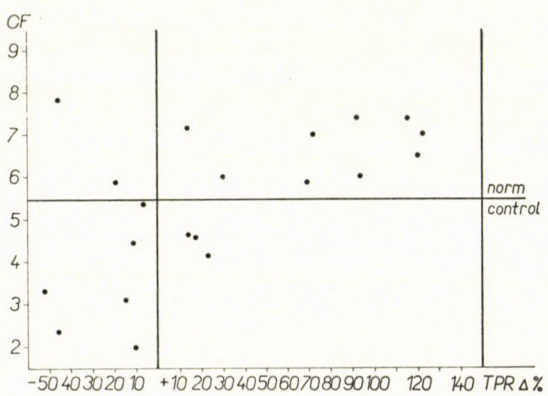

Fig. 3

sure or the other parameters (Figs 6, 7, 8). Solely the TPR seemed to be correlated to a certain extent with coronary flow (Fig. 7).

As to the occurrence of shock in dependence on the extent and site of myocardial damage, gross and microscopic findings failed to reveal any such connection.

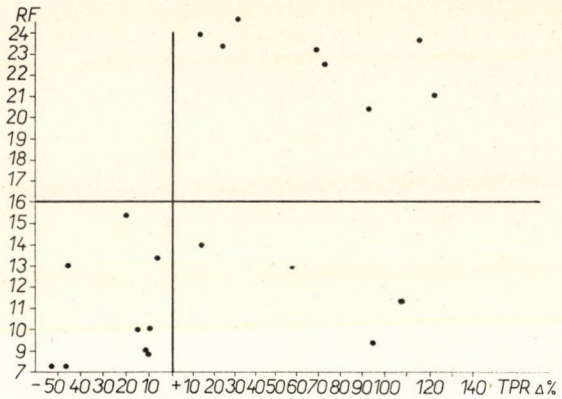

Fig. 4

Table II

No.		CO	Δ%	TPR cm dyne sec^{-5}	Δ%	BP mm Hg	Δ%
1.		5.72		2 510		180	
	30′	4.09	−29	2 350	− 6	120	−33
	60′	3.65	−36	1 670	− 34	75	−58
2.		3.16		3 680		180	
	30′	1.94	−39	6 650	+ 81	160	−11
	60′	1.42	−55	8 200	+123	190	0
3.		2.98		4 800		180	
	30′	1.71	−43	7 500	+ 56	160	−11
	60′	1.01	−66	14 200	+196	180	0
4.		2.88		4 150		170	
	30′	2.01	−30	5 550	+ 33	140	−18
	60′	1.83	−36	6 100	+ 47	140	−18
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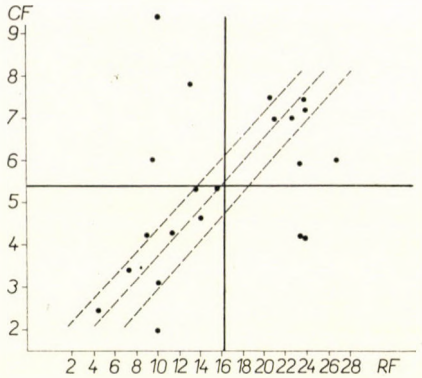

Fig. 5

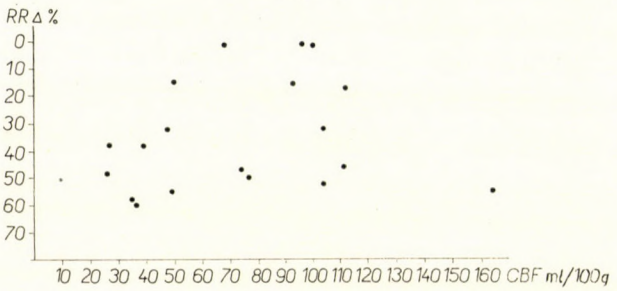

Fig. 6

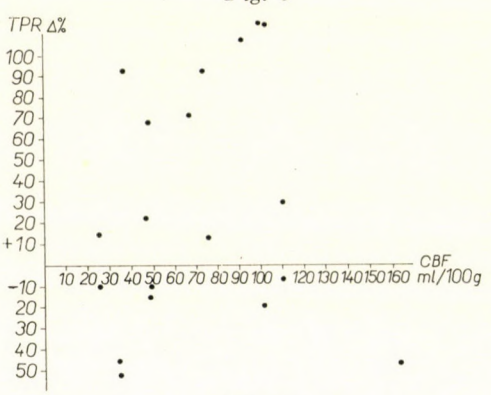

Fig. 7

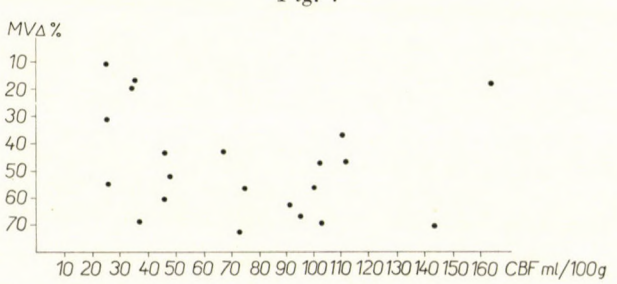

Fig. 8

Discussion

Experimental myocardial infarction whether or not inducing shock, was associated with a significant reduction of cardiac output in nearly every case, in agreement with most other observations relating to cardiogenic shock [6, 7, 8, 9].

The experiments designed to induce shock with a considerable drop of blood pressure were successful in 2/3 of the cases. In these instances as well as in those where no shock ensued, cardiac output fell in a similar manner. It was, however, the behaviour of TPR which proved distinctive of the two groups. A considerable rise in TPR was noted in those cases where no shock occurred, in contrast to those associated with shock, these being accompanied by an insignificant, if any, rise or occasionally, a reduction, of TPR. AGRESS et al. [3, 6, 10] have likewise found cardiogenic shock to occur if the TPR fails to rise, or fails to do so sufficiently. It is to be noted that in some of our experiments a reduction of TPR was registered.

TPR is known to reflect the changes in calibre of the peripheral vascular bed. In those cases where no shock occurred, it may be assumed that the drop of cardiac output was compensated by peripheral vasoconstriction, therefore no incongruity could arise between the peripheral vascular bed and the volume of circulating blood. In the cases with shock, the vasoconstrictive mechanism compensating the fall of cardiac output was either inadequate, as in the cases where its rise was insignificant, or entirely absent, in the cases where no change occurred.

In the face of this evidence we may well ask whether the behaviour of TPR reflects three different kinds of shock mechanism or merely three phases of one and the same process. It is an open question whether any distinction should be made between the group considered by AGRESS as shockless, i.e. where a reduction of cardiac output but none of the blood pressure occurred, and the one with manifest shock. On the evidence obtained in Group II, blood pressure is restored to normal within one hour in the majority of the cases, but this does not exclude the possibility of shock at some later stage. This issue could not be explored within the scope of the present experiments though being of primary importance since a prevalently vasoconstrictive or conversely, a vasodilatory mechanism, obviously requires entirely different therapeutic measures. The view that the changes in TPR reflect different phases of the same process gives us the correct therapeutic attitude in adapting the necessary measures to the changing features of the clinical picture even if this should involve the use of oppositely acting drugs.

One of the objects of the present experiments was to determine coronary blood flow. This was reduced in all of the cases even in those where there was a rise in the coronary fraction of cardiac output. Our studies failed to confirm

the existence of a linear correlation found by CORDAY et al. [11] between blood pressure and coronary blood flow. In opposition to the findings of these authors, in our experiments a 40 per cent drop of coronary flow ensued even in those cases where blood pressure remained unchanged.

Coronary vascular resistance increased in 4 cases and remained unchanged in 5. The value of this observation is made questionable by the possibility that the very method by which myocardial infarction has been produced, the injection of globules into the coronary bed, may be responsible for the rise in coronary vascular resistance. In 11 cases, however, that is, in the majority, a decrease in coronary vascular resistance, in other words, coronary dilatation has been registered. This means that a reduction of coronary flow occurred despite the coronary dilatation and of the frequently increased coronary fraction of cardiac output. This permits to link up the reduction of coronary flow in myocardial infarction with the fall of cardiac output. It is, however, another question whether the mechanism of this reduction is prevalently central or peripheral.

Neither vasodilatation nor the rise of coronary fraction was sufficient to compensate for the insufficiency of coronary flow. Another point studied was the behaviour of renal flow. This was found to increase or to diminish parallel with the respective changes in TPR, which signifies that the period of 5 minutes following coronary obstruction was in all likelihood too short for a characteristic shifting of cardiac output.

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NOCTURNAL ENURESIS

ELECTROENCEPHALOGRAPHIC AND CYSTOMETRIC EXAMINATIONS

By

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In patients with nocturnal enuresis a slowing of the waking spontaneous EEG, and occasional epileptiform activity have been observed. Induced sleep activated central and antero-frontal spike discharges in nearly 50 per cent of the cases, in some patients a spike-and-wave pattern or temporal foci appeared. In 77 per cent of the patients, enuresis was evoked by anaesthesia alone, in 9 per cent exclusively by sensory stimuli applied under the narcosis, while in 14 per cent such attempts failed to precipitate enuresis. In 83 per cent enuresis coincided with the phase of barbiturate anaesthesia characterized by delta-activity. The same depth of narcosis at which enuresis occurred, increased the number of spikes in 14 per cent of the cases only, while in the other cases it was associated with a suppression of spike activity. Epileptogenic cerebral injury was rarely demonstrable but in 55 per cent of the cases there were definite signs pointing to cerebral lesion due to injury at birth or to some other cause.

It is assumed that a developmental immaturity of certain non-specific structures situated in the primary sensorimotor area and the antero-frontal cortex, as reflected by a central or frontal spike activity, is a significant factor in the pathogenesis of nocturnal enuresis.

Our recent investigations into the paroxystic disturbances of autonomous visceral innervations have led to the study of the bioelectric abnormalities of the brain associated with the syndrome of periodic abdominal pain [21] and with nocturnal enuresis. The present paper deals with the latter problem.

ÓVÁRY, EGERVÁRY and ZSADÁNYI [25] have submitted to their special test 50 young soldiers with nocturnal enuresis. In 22 they could provoke enuresis by hexobarbital anaesthesia alone or by its combination with suprapubic cooling. Parallel EEG tracings recorded in 8 cases showed enuresis to coincide with the phase of deepening anaesthesia characterized by delta-activity. In other respects the tracings have not been studied. A modification of this procedure has been adopted for current use at our Department. A positive response, i. e. enuresis ensuing either spontaneously under general anaesthesia or upon provocation, has been interpreted by ÓVÁRY et al. to point to an organic releasing factor, while in case of a negative response they incriminate psychogenic functional factors.

EEG changes in enuretic children and juveniles have received little attention [3, 12, 15]. The investigations of TURTON and SPEAR [30] deserve special interest. "The EEGs from 100 cases of severe enuresis but without any discoverable organic condition were studied . . . Only 26 of these were completely normal . . . 28 showed a too slow or immature type of record . . . 22 in all,

were of an epileptiform type with 14 of these showing typical seizure patterns either at rest or during activation procedures . . . Two showed a focus in the left temporal lobe . . . There was one . . . with a well marked delta-and-spike focus in the left parietal lobe." Rolandic spikes are not mentioned in this study and recordings were made only in the waking state.

The enuretic type of petit-mal epilepsy has been described by GASTAUT, ROGER and FAVEL [9]. Simple diurnal and nocturnal enuresis was associated in 4 cases with bursts of 3 c/sec wave and spike activity. In 9 cases, the spells of absence were followed by voiding of urine either during the myoclonic bursts or at the end of the spells. VIZIOLI observed 5 cases of petit-mal with enuresis [31].

Material and methods

A total of 185 patients was investigated for nocturnal enuresis at our Department, 56 (30 per cent) females and 129 (70 per cent) males. This sex distribution was in close agreement with that observed by TURTON and SPEAR, i. e. 37 per cent females and 63 per cent males. According to HALLGREN [13] "the morbidity risk for nocturnal enuresis is significantly higher in the male sex". No juveniles enlisted for military service were included in the material. The age of the patients ranged between 5 and 57 years; those over 16 amounted to 16 per cent (29 cases), those between 7 and 13, for 67 per cent (118 cases), the age group between 12 and 13 for 21 per cent (40 cases), while that of 5 to 6 years for 7 per cent (12 cases). In contrast, TURTON and SPEAR found a prevalence of the group between 5 and 6 years (28 per cent) while those over 16 accounted for 8 per cent.

In our cases, both diurnal and nocturnal, enuresis, was confined to a small group of children while in the material studied by TURTON and SPEAR such cases were prevalent.

By means of urologic and neurologic examinations it was possible to exclude from the study the cases with enuresis of extracerebral origin and also mental defectives.

1. EEG tracings were recorded with the patient in the supine position. The majority of the records were made with a 1955/56 type 8-channel Kaiser apparatus, in the last two years a 16-channel Galileo apparatus was used. Anterior frontal, central, parietal, antero-, medio- and postero-temporal electrodes were placed symmetrically to identical sites as well as in the midline to the vertex forming sagittal and coronal chains. Wherever possible, nasopharyngeal electrodes were also used. In some cases, monopolar tracings were also made using average electrode.

Routine recording in the resting waking state included two 3-minute continuous records interrupted by a 3-minute run under hyperventilation and terminated by photic stimulation by means of white light with flashes at various frequencies.

2. After termination of the waking EEG, in 122 patients the emptied bladder was filled under sterile conditions with a permanganate-stained 0.85 per cent saline solution until definite urgency of micturition was felt but no actual voiding occurred. Thereafter the catheter was removed and collection of urine was provided for.

In 63 patients intravesical pressure was measured by means of a water manometer attached to the tip of the catheter with its 0-point at the level of the symphysis. The waking resting value was registered, and EEG and cystometric recording was continued during hexobarbital anaesthesia. Hexobarbital was injected intravenously at a rate of 25 mg/15—10 sec, depending on age, until continuous delta activity had become manifest over the whole brain, a pattern which on close analysis of a great number of tracings has been found to reflect a condition of narcosis of medium depth [20]. When this alone failed to provoke enuresis or a corresponding rise in intravesical pressure attaining 40 cm water, the abdominal skin of the suprapubic area was cooled with chlorethyl or ether. If this too failed, pressure with the palm was applied to the suprapubic region for 20 to 30 sec. During anaesthesia repeated sensory stimuli were applied such as electric buzzer, calling by name, clapping of hands, pricking, pinching. Timing of these stimuli was carefully checked. Nociceptive stimuli were applied to the skin of the arm.

EEG and cystometry were repeated in 13 patients on one to three occasions after an interval of several days provided the previous test had not been followed by cystitis.

3. Background activity of the resting waking EEG was assessed by the standards laid down by GIBBS and GIBBS [7] and by FOIS and LOW [5]. Over the age of 5 years, spontaneous high voltage delta-runs were considered pathological. The more so, the greater was the prevalence of this activity over other spontaneous rhythms and also in relation to age. The appearance of groups of 5—7 c/s was considered physiological up to the age of 7, slight slowing between 7 and 10 years, and, in case of persistent recurrence, as a moderate abnormality beyond 10 years of age.

All in all, 202 recordings were made in the 185 patients.

Results

I. 1. Spontaneous activity was considered normal in 48 per cent (89 patients), slightly or moderately slowed in 33 per cent (61 patients) and definitely slowed in 19 per cent (35 patients).

2. Spontaneous epileptic activity was demonstrable in 5 per cent (9 cases), i.e. fronto-central spike focus, 2 cases; antero-frontal, 1 case; central, 2 cases; parieto-temporal, 2 cases and generalized spike-and-wave variant, 2 cases.

Burst or spike activity was evoked by photic stimuli in 4 cases (Fig. 2).

3. Activation of seizure potentials by hexobarbital anaesthesia was demonstrable in a number of cases (see Table I).

It was thus possible to identify spike discharges or other epileptiform patterns in 47 per cent of the tracings recorded during induced sleep. This incidence was considerably higher than in the 100 cases analyzed by TURTON and SPEAR, a divergence obviously due to the higher informative value of records made during induced sleep, since both diurnal and nocturnal, enuresis, was confined to a small group of our cases while the material studied by TURTON and SPEAR was largely made up of this severe type of enuresis.

Table I

	Cases	
	No	Per cent
Spikes in the central and/or frontal areas	53	56.9
Unilateral antero-frontal spikes	7	7.5
Bilateral antero-frontal spikes	5	5.3
Unilateral central spikes	4	4.2
Antero-frontal spikes and mittens	5	5.3
Unilateral fronto-central bursts and mittens	5	5.3
Generalized or focal epileptic pattern	5	5.3
Unilateral temporo-frontal spikes	3	3.2
Bilateral or unilateral baso-temporal spikes	3	3.2
Spike-and-wave pattern + antero-frontal spikes	2	2.1
Total	92 EEGs	

II. The timing of wetting was studied in relation to the bioelectric wave patterns marking the successive phases of hexobarbital anaesthesia [20]. Where intravesical pressure was measured, it was the initial phase of the steeply ascending curve, and in the other cases, the appearance of the first portion of urine which was regarded as the introductory phase of detrusor activity resulting in discharge of urine.

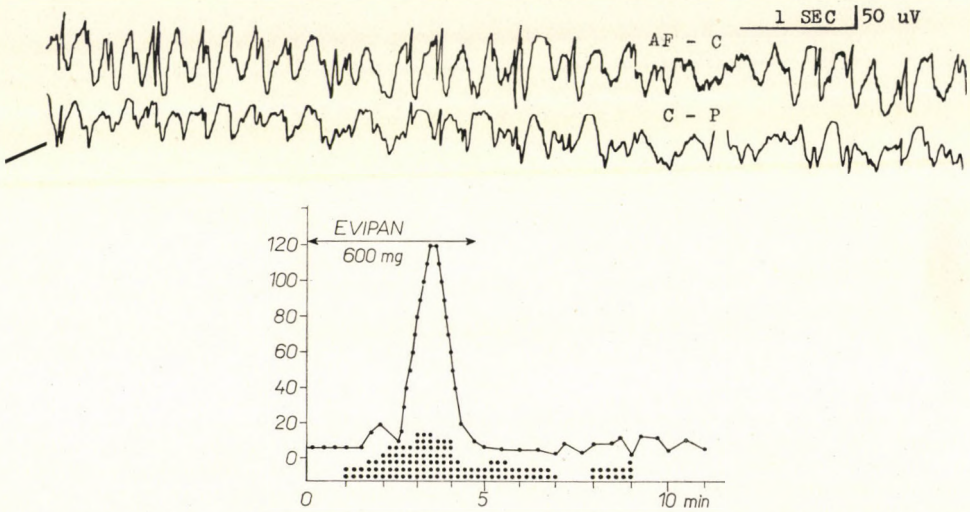

Fig. 1—1a. A 15-year old boy had had sustained a cranial injury at 18 months of age. He had had enuresis for several years. Recurrent spells of absence, on one occasion with falling, were experienced in the last year. Violent, ungovernable personality, poor school reports. PEG shows a dilated right lateral ventricle, the EEG displays a generalized 3 to 4 c/s spike-and-wave-pattern (Fig. 1), activated by hyperventilation; AF = antero-frontal, C = central, P = parietal electrode. — Enuresis test: intravesical filling with 140 ml of saline elicits a distinct urgency of micturition. It may be seen on Fig. 1a that intravesical pressure reached its peak in the 4th minute after beginning the injection of hexobarbital, parallel with the maximum of spike-and-wave activity. The dots over the abscissa represent each a spike discharge

1. The beginning and the end of the sharp rise in intravesical pressure, whether spontaneous or induced, were found to span 60 to 150 seconds, rarely exceeding the upper limit of this period. The duration of actual micturition varied between 25 and 65 sec. Voiding of urine or the pressure phase occurred prevalently between the second and fourth minute after starting the barbiturate injection.

2. The background EEG registered in 148 cases during the phase of 10 seconds preceding immediately the discharge of urine (or the abrupt rise in pressure) could be classified into the following morphologic types (see Table II) on the basis of measurements of the periods occupied by one or more delta-runs.

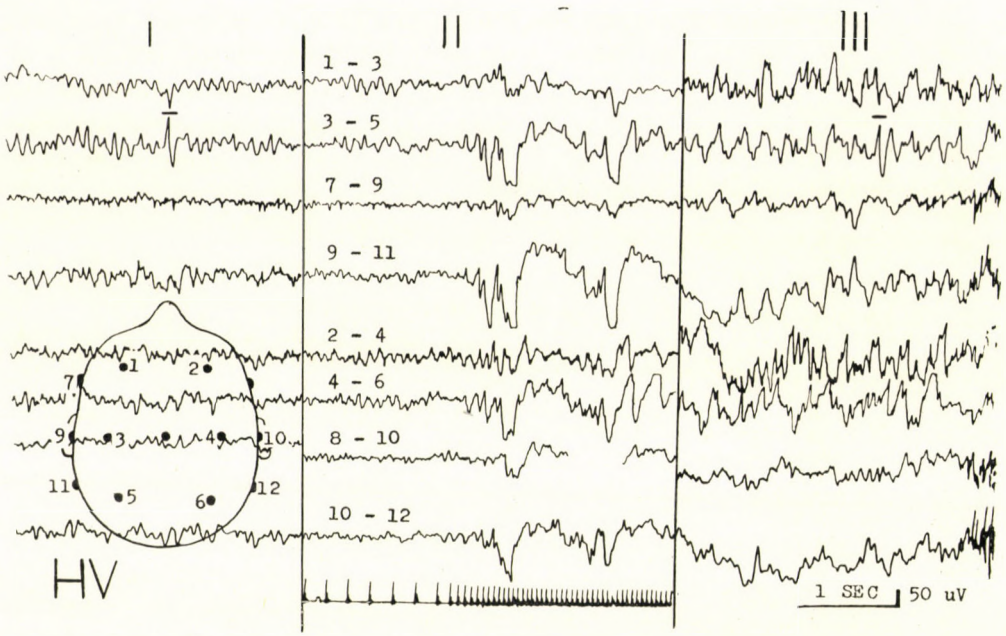

Fig. 2. This 12-year aged girl had a dry period between her first and 3rd year, until she underwent a severe influenza. From this time on she has bedwetting every night. She was a heavy sleeper. Nervous, irritable behaviour, mediocre school records. During glutamic acid treatment, wetting occurred twice a week only. Part I of Fig. 2: Waking record made during hyperventilation, which provoked central spikes over the left hemisphere (the spikes are marked by a short horizontal line). Part II: Burst-activity over the left centro-temporal area provoked by 20 c/sec flickering light. Part III: Detail of tracing recorded in the 2nd minute of barbiturate administration during the rise of intravesical pressure. This also shows spike discharge at the left side

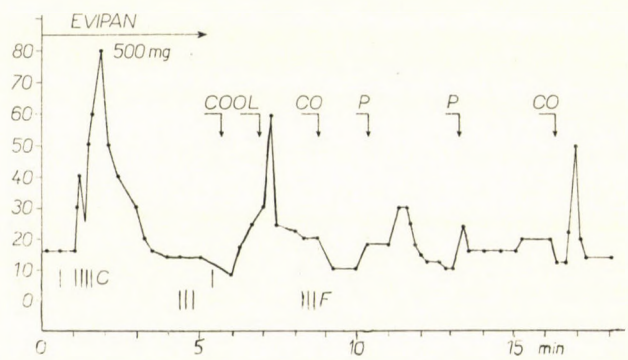

Fig. 2a. Spontaneous intravesical peak pressure in the 2nd to 4th minute of barbiturate anaesthesia and the rise in pressure, slighter in degree and shorter in duration, elicited by repeated cooling or pressure on the suprapubic region. Each of the vertical lines over the abscissa mark a spike discharge. In the initial phase of barbiturate effect spikes appear over the central area only, and later almost exclusively over the frontal pole. C = central, F = frontal, COOL = cooling, CO = pressure, P = pricking

As data in Table II show, in 83 per cent of the tests enuresis tended to coincide with that phase of deepening anaesthesia in which the delta-runs either represented the predominant activity or showed a steadily increasing tendency. This is in line with the assertion of SCHIFF [28] that nocturnal enuresis is unrelated to the rapid eye movements (REMs) marking the period of sleep, this being characterized by a low-voltage, rapid EEG pattern.

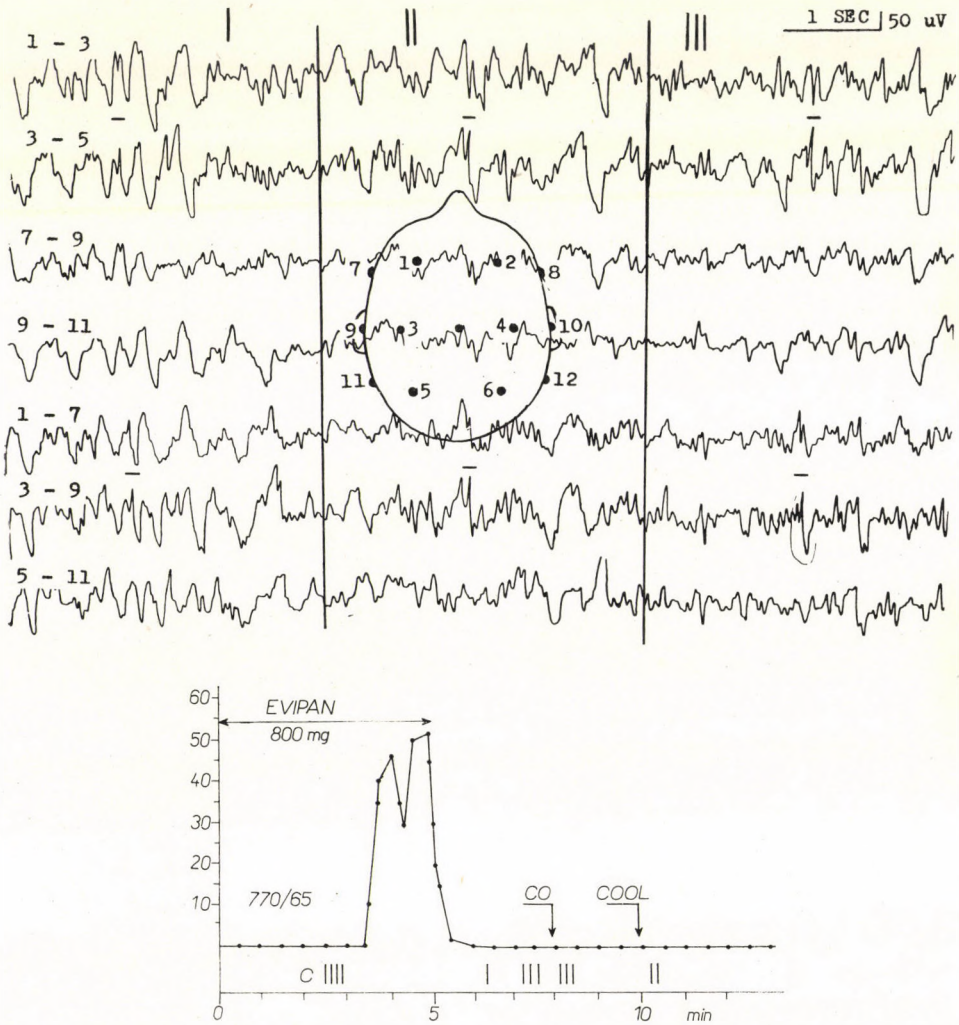

Fig. 3—3a. Male, aged 19 years. Complicated delivery. He has had enuresis since the age of 2 years. The frequency of wetting has been declining. He has always been a heavy sleeper with no intellectual or behavioural abnormality. The PEG shows ampler filling over the left hemisphere. The waking EEG is characterized by a moderate slowing of spontaneous activity; under hexobarbital anaesthesia negative spike-discharges of left rolandic origin (Fig. 3). Enuresis test: Definite urgency upon filling with 200 ml saline. During anaesthesia, spontaneous intravesical pressure-peak becomes apparent while, parallelly, the spiking ceases (Fig. 3a)

Table II

	Cases	
	No	Per cent
Prevalence of delta-waves or full delta-activity	85	57.4
Delta-runs in increasing number	38	25.6
Delta-runs still absent	23	15.5
Mixed background activity of theta-alpha-beta-groups just before awakening	2	1.3
Total	148 EEGs	

SCHIFF [28] has furthermore found enuresis in children to be associated with a slow, high-voltage EEG pattern in contrast to that of adults which is accompanied by a bioelectric activity of higher frequency and lower amplitude. This agrees well with our observations in induced sleep. Nevertheless, in 20 of our cases the event of enuresis was associated with a more or less marked reduction in voltage and increase in frequency before delta-activity

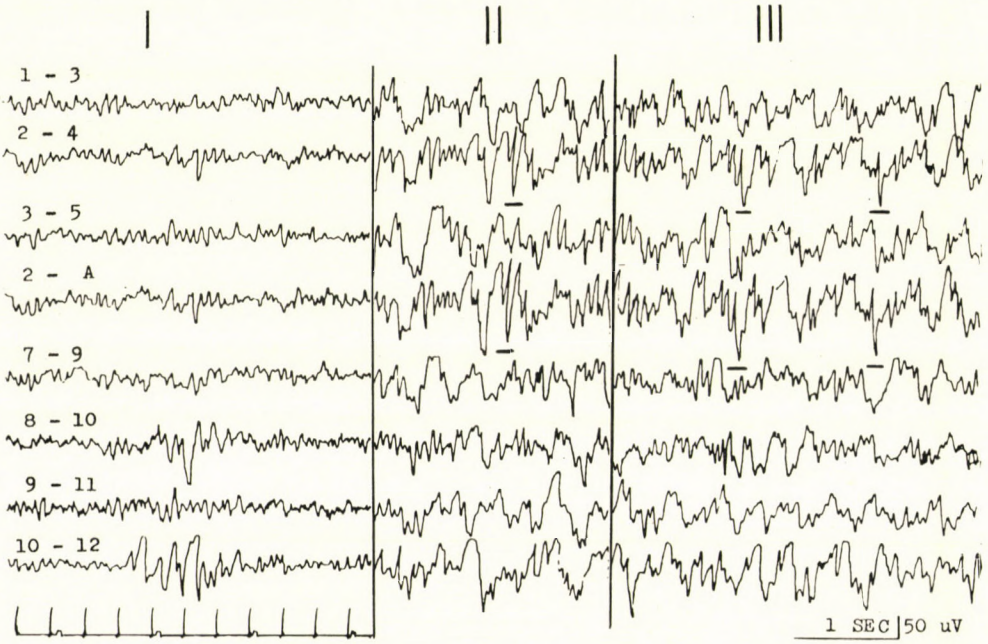

Fig. 5. Male, aged 12 years, normal delivery. Had suffered a cranial injury at the age of 7. Enuresis of increasing frequency from 6th year onward, recently this has been occurring night after night invariably during a heavy sleep. Nervous personality, poor school reports. Epileptic mother. A sibling was enuretic until adolescence. Part I: Burst activity precipitated by photic stimuli prevalently over the right temporal region. Parts II and III: Negative spikes appear over the right frontal pole during spontaneous micturition in the 4th minute of induced sleep. Lead A recorded by unipolar lead. Filling with 220 ml saline

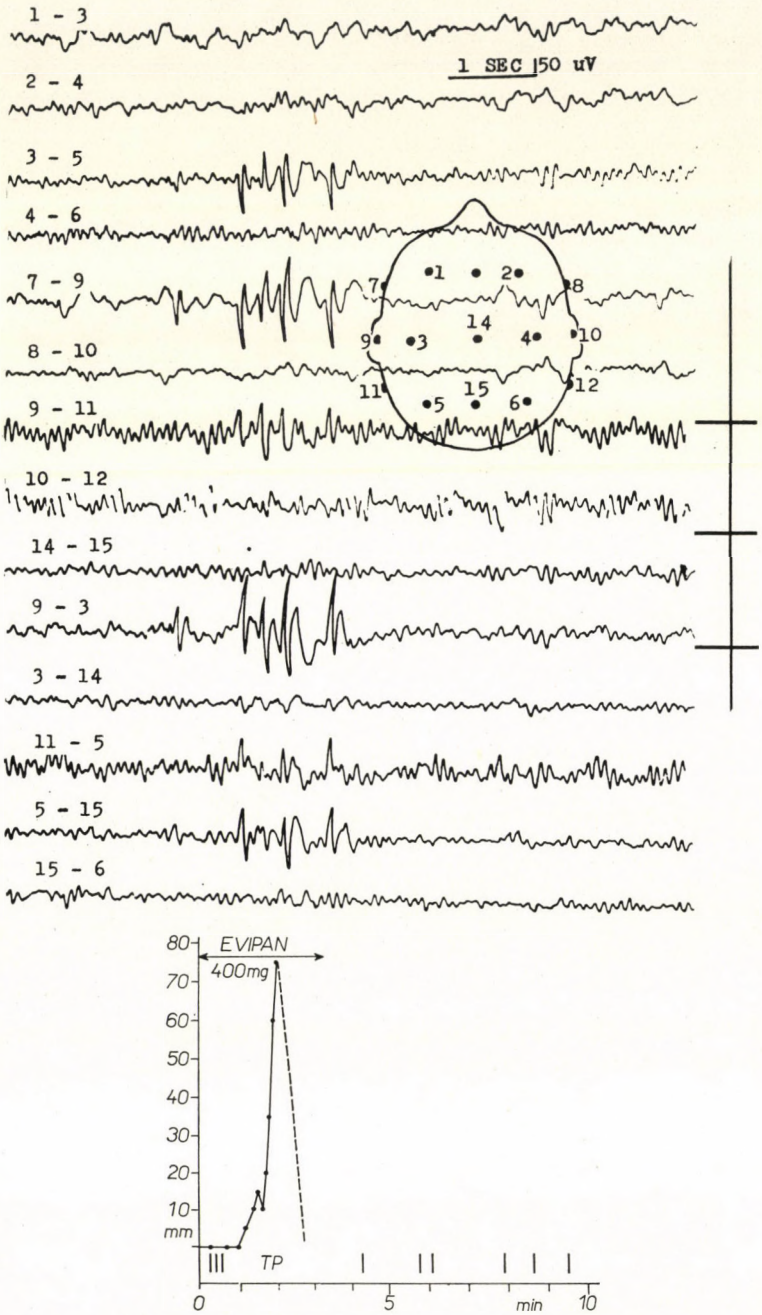

Fig. 4—4a. Male, aged 8 years, born in asphyxia, and needed protracted artificial respiration. He has never acquired control of the bladder. Heavy sleep; hypogonadism, normal intellect; slightly irritable. Fig. 4: Waking EEG. Spontaneous spike groups over the left temporo-parietal region. Fig. 4a: Enuresis test. Filling with 80 ml saline causes moderate urgency. Spontaneous intravesical pressure-peak resulting in micturition with spontaneous release of catheter. Suppression of spike activity during the phase of enuresis

had become predominant, while in 3 further cases partial desynchronization appeared in a continuous delta-activity. In these cases wetting was accompanied by spontaneous incoordinated movements and dilatation of the pupils the extent of which could easily be estimated.

3. Spontaneous enuresis occurred twice or more in 13 cases, during deepening narcosis or just before awakening.

After a positive enuresis test in 13 cases the repeated test was positive again in 11 cases.

Two siblings (not twins) displayed a response of identical pattern with ipsilateral spike activity in the central region.

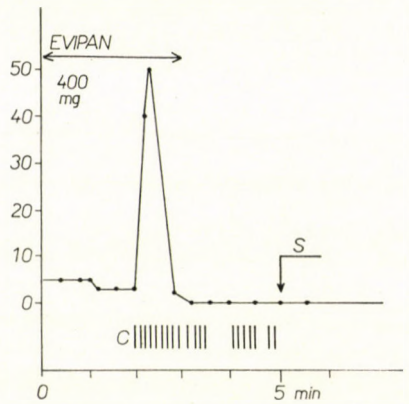

Fig. 6. Male, aged 12 years, has been enuretic since the age of 3. The frequency of wetting, invariably during heavy sleep, has been increasing. No cerebral lesion in the history. Normal physical and mental development. On filling the bladder with 160 ml saline, urgency of moderate intensity. In the 3rd minute of barbiturate injection a spontaneous intravesical pressure peak appears and a contemporaneous spike activity becomes manifest over the central area, its maximum frequency per minute coincides with the detrusor contraction. Auditive stimuli (S) have no effect, but spontaneous miction occurs just before awakening

In 25 cases (13.5 per cent) neither spontaneous nor induced enuresis was observed during deepening sleep.

4. In 17 cases (9 per cent) enuresis could only be elicited by suprapubic cooling or pressure. The precipitating influence on enuresis of cooling, pressure, furthermore of intermittent auditory and painful stimuli was compared in 50 cases. Applying the stimuli over 20 to 30 sec, it could be ascertained that the effect of compression was equivalent to that of cooling, moreover that both were apt to elicit enuresis far more frequently than did either auditive or painful stimuli (Table III).

During the initial phase of barbiturate anaesthesia, reduction of intravesical pressure by 3 to 10 cm may occur (Figs 2a and 7). Sensory stimuli applied during sleep may also result in a slight reduction of intravesical pressure which is either followed by an abrupt rise (discharge of urine) or by a return to

Table III

Stimulus	Number of stimulations	Number of stimulations resulting in enuresis	Number of ineffective stimulations	Number of patients
Suprapubic cooling	43	13 (30%)	30	38
Suprapubic pressure	45	13 (30%)	32	39
Auditory stimulus	21	2 (10%)	19	16
Painful stimulus	13	1 (8%)	12	10
Total	122	29 (24%)	93	50

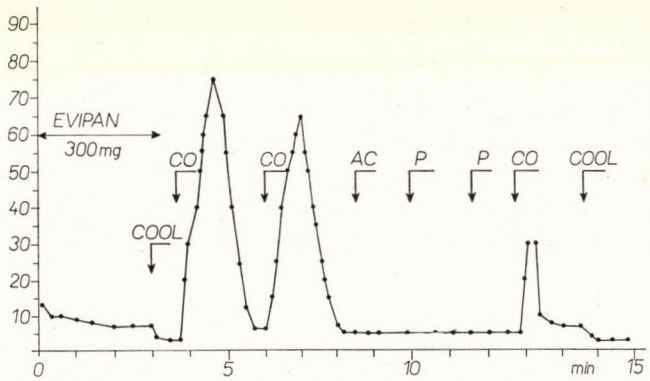

Fig. 7. Male, aged 8 years. Normal delivery. Enuresis exclusively at night; he had never had a dry period. In the last two months there has been occasional encopresis; no neurologic or intellectual abnormality. Filling of bladder with 85 ml saline, induced sleep causes no enuresis. Cooling elicits reduction of intravesical pressure on repeated occasions, while suprapubic pressure invariably precipitates the intravesical pressure-peak though in a progressively decreasing degree as sleep becomes less deep. Induced sleep fails to evoke convulsive potentials

the initial pressure value (Figs 2a and 7). In some patients the enuretic response was elicited by a sole sensory stimulus invariably of the same kind (Fig. 7).

III. The tracings recorded during sleep in 35 persons under 15 years of age were studied for the correlations between peak intravesical pressure and the appearance of seizure potentials. These tracings were picked out at random from those showing epileptiform discharges.

1. In the majority of the patients (26, i.e. 74.8 per cent) no or scarcely any seizure potentials could be recorded in the phase of anaesthesia coinciding with the peak of intravesical pressure or with micturition. Often the convulsive activity totally ceased during enuresis while just before and after it spike discharges appeared (Figs 2a, 3a, 6). In opposition to this, phase of sleep by which the actual micturition (i.e. pressure peak) is ushered in, was found to be optimal for seizure potentials (Figs 1a, 6). In 3 cases the timing of the spike

discharges seemed to be unrelated to the phase of sleep characterized by the onset of enuresis. In one case, the spikes activated by hexobarbital were recorded over the central region before intravesical pressure had reached its peak while the longer section of the tracing subsequent to the peak exhibited — with one exception — antero-frontal spikes only (Fig. 2a).

2. In an earlier study [21] it has been shown that in recurrent abdominal pain paroxysms of cerebral origin 65 per cent of the spike potentials activated by barbiturates were of rolandic origin. Incriminating for nocturnal enuresis a similar cerebral mechanism involving the autonomous visceral innervations, we found it pertinent to establish the ratio of spike-bursts identifiable over the central area, the EEG tracings of 30 cases from the foregoing group of 35 patients were analyzed.

The prevalent focal spike activity was recorded over the central region in 18 cases, over the antero-frontal region in 9 cases, while in 3 cases both antero-frontal and central epicentre were found to discharge with approximately the same frequency. In 437, i.e. 61 per cent, out of 717 confirmed spikes the peak voltage was recorded over the central area, and in 280, i.e. 39 per cent, over the frontal pole. The ratio of rolandic spikes corresponded to that found in the cases with periodic abdominal syndrome.

IV. The antecedents of the first hundred patients studied by us permitted to estimate the frequency of cerebral lesion sustained before the appearance of nocturnal enuresis [26]. The history was suggestive of birth injury in 31 per cent, of cranial injury of some other kind in 16 per cent, while in 8 per cent the history revealed earlier meningoencephalitis. In 16 cases, information given by the relatives was inadequate, while in 18, any possibility of cranial or cerebral injury was denied. In sum, earlier cerebral lesion had occurred in 55 per cent of the cases.

19 persons of the same group had acquired transient control between the age of 2 and 5 years, but the 81 other patients never had dry periods. Clinical epilepsy was presumably present in 3 cases.

Discussion

Certain facts emerging from the present observations may be linked up with the pathogenesis of nocturnal enuresis.

a) Releasing of enuresis by induced sleep is certainly an abnormal reaction. Barbiturate anaesthesia, in the same way as spinal transection or blockade of the sacral roots or even death, fails to cause contraction of the detrusor musculature, i.e. discharge of urine, in cats (Ruch [27]). In barbiturate poisoning the loss of the micturition reflex in the comatose patients is common. The observation that enuresis has been precipitated by barbiturate anaesthesia in our patients with enuresis would therefore suggest an inadequate func-

tion of the corticospinal inhibitory mechanism which under normal conditions is controlling the detrusor contraction in both natural and barbiturate sleep. The cause of this insufficiency of the inhibitory corticospinal impulses transmitted through sympathetic pathways may lie in a developmental immaturity.

Cerebrocortical influence on the functions of the bladder has been substantiated by experimental and clinical evidence. The "centre" of bladder control has been located in humans to the primary sensorimotor area, the paracentral lobule. Recently, it has been demonstrated by GJONE and SETEIKLEV [10] using electric stimulation that the act of micturition in cats is controlled by excitatory and inhibitory effects arising from the primary and secondary sensorimotor areas. Regulatory impulses to the vesical musculature in cats are furthermore emitted by the gyri, cinguli and orbitalis, the pyriform cortex and the amygdaloid complex (KAADA [18], GJONE [11]). Various descending cortical influences are transmitted through hypothalamic pathways and many of the hypothalamofugal impulses are mediated by the brain-stem reticular formation (MORIN [24], CROSBY and WOODBURNE [2]).

b) Spontaneous filling of the bladder elicits an urgency of micturition arousing the healthy, but not the enuretic, child from his sleep. 40 per cent of the enuretic children studied by HALLGREEN [13] were heavy sleepers. This is in agreement with our own observations. According to the, mostly spontaneous, reports of relatives, an unusually heavy sleep was characteristic of a great number of children. Some parents even noticed that voiding coincided with the deep phase of sleep. This would implicate a delayed maturation of the arousal mechanisms of the brain in nocturnal enuresis, a claim consistent with our finding that association of enuresis with partial desynchronization of EEG background activity and signs of the diminishing depth of anaesthesia occurred in 14.8 per cent of our patients only. Moreover, there are definite indications derived both from animal experiments [6, 19] and from studies on humans [22] that the primary sensorimotor cortex, a "corticoreticular" area in a sense, has arousal functions. On these grounds, immaturity of the arousal functions of the "corticoreticular" structures may well be one of the significant factors accounting for nocturnal enuresis.

This possibility is supported by the fact that more than 50 per cent of the spike discharges were recorded over the primary sensorimotor areas.

GASTAUT [8] ascribes the spikes in spastic paresis to a prerolandic hyperexcitability due to a bombardment by the continuous inflow of proprioceptive afferent stimuli. A mechanism of this kind does not enter into consideration in our cases, since sensorimotor functions were normal in all of our patients. Nor does the corticopetal sensory stimulus by miction account for the rolandic spike activity, since it was during the very phase of enuresis that this activity ceased. The central spikes could not have been due to the specific somatomotor or sensory elements of the central cortex either, since partial motor or sensory

epileptic manifestations were rare in the subjects with enuresis. The rolandic irritative foci in children have been interpreted by BANCAUD, COLOMB and DELL [1] as being due to a peculiar response pattern of the central regions characteristic of definite stages of maturation. ISLER and HESS [16] found a prevalence of central foci in the 2 to 8 year age group.

The rolandic spikes registered in our cases originate in all likelihood from those nonspecific structures of the central regions which emit descending regulatory impulses to the micturition reflex on the one hand, and to the reticular arousal structures of the brain-stem, on the other. Negative spikes recorded over the central area may, however, represent projections of an activity originating in subcortical structures. FAETH and WALKER [4] could produce chronic epileptogenic foci by injections of alumina cream into the globus pallidus, the putamen, the thalamus and the amygdala. These foci were found to generate spikes or sharp waves in the ipsilateral central area.

Therefore in certain cases a spike activity recorded over the central area may possibly originate from some of the basal ganglia. There furthermore exist, in all probability, connections between the basal ganglia and the function of the bladder. According to clinical observations (TÖNNIS and BISCHOF [29]) pathologic processes involving these structures may affect the innervation of the bladder over periods of several years.

A focal negative spike activity recorded over the frontal pole also belongs to the abnormal patterns, its pathological significance is, however, unclear. These spikes may represent a sign of immaturity of certain inhibitory functions of the frontal cortex, since lesions of the antero-frontal area have been found to cause, in the majority of cases, functional disorders of the bladder, particularly urinary incontinence (KOLODNY [23], HOFSTÄTTER [14]). The frontal pole seems to exert inhibitory functions on behavioural patterns (JARVIE [17]), structural lesions of the frontal pole being associated with a behavioural disinhibition, including a disinhibited micturition reflex.

As an explanation of the coincidence of enuresis with increased spike activity in some of our cases, it may be alleged that the efferent inhibitory influences on micturition may have been blocked by the epileptiform discharges either directly or indirectly through the activating influence of induced sleep on convulsive potentials or by both. As another alternative, both enuresis and the parallel appearance or increase of epileptiform activity might be ascribed to discharging non-specific cortical structures with a stimulatory activity on enuresis.

Nocturnal enuresis is rarely associated with a temporal or basotemporal convulsive activity; this indicates that the neural structures of the human temporal lobe have little influence on the micturition reflex.

d) The possibility of inducing enuresis by peripheral sensory stimuli also support the claim that the mechanisms inhibiting the micturition reflex

are inadequate or immature. Voiding elicited by suprapubic irritation is probably the result of a simple segmental spinal reflex mechanism, since response confined to a sole sensory modality presumes an enhanced reflex irritability. It has been noted by RUCH [27] that emotional reactions are accompanied by a definite rise in intravesical pressure while coughing, straining at defecation or micturition have no such effect in the same individual.

e) In the majority of our cases, there may have been a causal relationship between cerebral injury and immaturity of the cerebral functions controlling the reflex of micturition. Such a delay is generally overcome in the course of cerebral and hormonal maturation during adolescence, provided no epileptogenic foci or mental defect have persisted.

f) The present observations have a certain practical interest, since in nocturnal enuresis of cerebral origin, by restriction of fluid intake during the second half of the day, coupled with a combination of non-hypnotic anticonvulsant and drug capable of reducing the depth of sleep, we may succeed in reducing the frequency of enuresis or even to bring it under control, particularly in cases associated with an excessive depth of sleep and an epileptiform EEG activity [26].

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THE IN VITRO METABOLISM OF DEHYDROEPIANDROSTERONE IN HUMAN SKIN*

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Segments of human skin were removed surgically from the pubic and abdominal areas of female patients. Skin-slices were incubated *in vitro* for 5 hours with Krebs-Ringer-phosphate-glucose media containing dehydroepiandrosterone-4-^{14}C. A number of labelled products were detected by means of column (Al_2O_3), thin-layer (Al_2O_3, silica-gel G) and paper chromatographic techniques. ^{14}C-labelled androst-4-ene-3,17-dione, 7α-hydroxydehydroepiandrosterone, 7-ketodehydroepiandrosterone and androstane-3,17-dione could be isolated from incubating media. The results of these incubations point to the fact that the skin contains enzyme-systems which are capable of metabolising dehydroepiandrosterone *in vitro*.

It is well known that structural and functional alterations in the skin are frequent manifestations of endocrinological disorders. For example, the brown-coloured hyperpigmentation of the skin in Addison's disease, the purple-coloured striations of the abdominal skin in Cushing's syndrome, the pale, dry, oedematous skin in hypothyroidism are characteristic attendants of these diseases.

In females, some of the symptoms of hyperfunction of the adrenal cortex (Cushing's syndrome, adrenogenital syndrome, etc.) or of the ovaries (Stein-Leventhal syndrome, arrhenoblastoma, etc.) appear on the skin as well. The skin is often "greasy", the sebaceous glands are overactive, blocking the pores and causing pyoderma and acne. These complaints are usually associated with hirsutism, moustache and beard development, and, depending on the type of disease, usually coarse hair appears on various parts of the body. These patients have very coarse pubic and axillary hair; the hair of the head is fine but dense and balding begins, according to the masculine pattern, on the crown of the head and at the temples. These observations seem to indicate that the hormone disorders are manifested not only in the skin but also in the hair.

Extensive blood and urine steroid tests conducted when studying the pathogenesis of hirsutism, have shown up a large number of steroid-metabolic disorders (BROOKSBANK 1961, DORFMAN 1963, JULESZ et al. 1963, DIGNAM et al. 1964, KORENMAN et al. 1965). However, we do not yet know to what extent

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these disorders contribute to the development of hirsutism. For this reason there is a great deal of uncertainty in the diagnosis and treatment of the condition. The majority of the published cases (BROOKSBANK 1961) and one-third of our own cases (JULESZ et al. 1963) had to be diagnosed as "idiopathic hirsutism", which confirms our scant information on its pathogenesis.

The endocrine relationship between human skin and hair growth has been the subject of numerous experiments and observations (HAMILTON 1960, STRAUSS and POCHI 1963, ROOK 1965, LEBON 1965, PAPA and KLIGMAN 1965, etc.). The human skin and hair are very rich in cholesterol (FAREDIN et al. 1966, TÓTH et al. 1966). We can detect a number of 17-ketosteroids, mainly dehydroepiandrosterone, in the skin and in the hair from different parts of the body (JULESZ et al. 1966a, b). These data also seem to bear out the assumption that androgenic steroids must play a part in the development of human hair. We assume that those women will suffer from hirsutism, whose pilosebaceous gland received an excess of androgenic steroid, causing a modification in the ratio of androgens to oestrogens. It is also possible that a change in the function of the enzyme systems in the skin around the pilosebaceous glands contributes to the development of hirsutism.

We know very little about those enzymes in the skin which metabolize steroids. According to the *in vitro* investigations of MALKINSON et al. (1959) and of HSIA et al. (1964, 1966) the skin may play a part in the metabolism of corticosteroids, but we have no data on the metabolism of androgenic steroids. Since human hair contains a considerable amount of dehydroepiandrosterone, it seemed profitable to find out whether the skin contains enzymes which can metabolize dehydroepiandrosterone further. Therefore, female skin-slices from different parts of the body were incubated *in vitro* with radioactive dehydroepiandrosterone and the radioactive products obtained were isolated by various chromatographic methods.

Materials and methods

Pubic (hairy) and abdominal (hairless) skin, obtained from surgery on female patients, was used. After trimming the specimens of adhering fat, the skin-tissue was sliced and the slices were weighed and incubated *in vitro*.

Method of incubation

The incubation media used were Krebs-Ringer-phosphate with 200 mg glucose/100 ml (KRPG), at pH 7.2—7.4. In addition, the media contained a final concentration of 10^{-3} M diphosphopyridine nucleotide (NAD) and in one case adenosine triphosphate (ATP). All steroid additions were taken up in 0.2 ml propylene glycol in the incubation flasks prior to the addition of the skin-slices. The DHA-4-^{14}C was obtained from the Radiochemical Centre, Amersham, 117 μc/mg and diluted with unlabelled DHA.

Skin-slices were incubated in a Gallenkamp metabolic shaker at 37 °C in KRPG media in O_2 atmosphere for 5 hours.

When, for control purposes, skin-slices were inactivated, this was accomplished by boiling the tissue slices in KRPG for 10 minutes, after which the slices only were transferred to a flask containing fresh medium and substrate for incubation.

Extraction and chromatographic procedures

All solvents were of BDH "Analar" quality and were redistilled before use.

After incubation for 5 hours, the KRPG media were decanted from the skin-slices. The skin-slices were first extracted with 3×5 ml of absolute ethanol and then left overnight in 15 ml acetone-water (2 : 1). The ethanolic extract was combined with the acetone-water extract and the solvents were evaporated. The aqueous residue of the pooled extracts was combined with KRPG media, the mixture was saturated with NaCl and Na_2SO_4 and extracted three times with 25 ml of methylene chloride and twice with 20 ml of diethyl ether. The pooled extracts were taken to dryness under a stream of filtered air at less than 45 °C.

The extracts were chromatographed on a 3 g alumina column (Merck A. G., Brockmann III/IV activity) 10 mm in diameter. Steroids were eluted from the column with stepwise increasing increments of ethanol in benzene according to FAREDIN et al. (1957). The individual eluates were pooled in I—VI fractions according to DINGEMANSE et al. (1952) (Table I).

Thin-layer chromatography of each fraction was carried out on alumina plates (Al_2O_3-G. Merck A. G. nach Stahl) using the systems of FAREDIN and WEBB (1966) and silica-gel-G (Merck A. G., Shandon) according to the system of LISBOA (1965). Localization of steroids was carried out with the Zimmermann and the $SbCl_3$ colour reactions and by absorption in ultraviolet at 240 mμ. The steroids were eluted from the thin-layer plates with 4×5 ml methanol. The recovery of steroids from the alumina plates was 85—100 per cent, and from silica-gel-G 70—90 per cent.

The eluates from alumina or silica-gel-G thin-layer plates were chromatographed on paper in the BUSH systems (1961). The paper was washed in a Soxhlet extractor with chloroform, benzene, ethyl acetate and methanol prior to use.

Detection and quantitation of radioactive steroids

Radioactive areas were located on paper or thin-layer chromatograms using a gas-flow Nuclear-Chicago 4π Actigraph II chromatogram scanner.

Radioactive steroid samples for quantitation, eluted from alumina, silica-gel-G or paper, were plated at "infinite thinness" and counted on an Isotope Developments Ltd. Flobetomat type 6051, thin endwindow, low background, automatic solid sample system operating at 21 per cent efficiency for ^{14}C.

Formation of steroid derivatives

Acetylations of isolated steroids were performed generally according to the procedure outlined by BUSH (1961).

Results

On Figure 1 Graph *A* shows that by the skin-slices DHA-4-^{14}C has been converted to several steroids *in vitro*. That these are true metabolic products of the substrate is shown in Graph *C*, in which it is seen that only DHA-4-^{14}C was recovered when this substrate had been incubated in the absence of tissues. Graph *B* indicates that when skin tissue had been inactivated by boiling before incubation no transformation of DHA occurred under the same conditions in the absence of active skin tissue.

Table II shows the results of incubation of skin-slices of two female patients. In the first case skin-slices were obtained from the pubic region only. One portion was incubated with ATP and the other, of much the same in quantity, was incubated without ATP. The radioactivity of the metabolites (Fractions I, IV, V, VI) was very similar. In the second case skin-slices from the pubic region as well as from the abdomen (hairless) were incubated. Although the

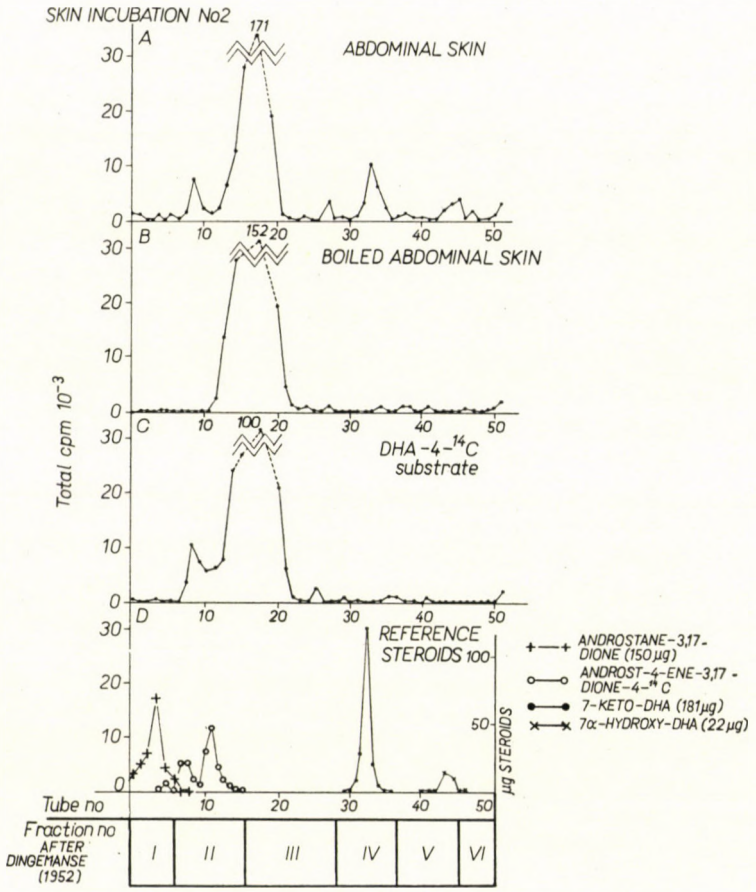
Table I

Alumina column chromatography according to FAREDIN *et al. (1957) and* DINGEMANSE *et al. (1952)*
Standard protocol

Tube No.	% Ethanol benzene	ml collected	Fraction No.	Tube No.	% Ethanol benzene	ml collected	Fraction No.
0	—	10		29	0.25	20	
1	—	10		30	0.25	20	
2	—	10	I.	31	0.25	20	IV.
3	—	20		32	0.25	20	
4	—	20		33	0.50	20	
5	—	20		34	0.50	30	
				35	0.50	30	
6	—	20		36	0.50	20	
7	—	20		37	0.50	20	
8	0.05	20					
9	0.05	20		38	0.50	20	
10	0.05	20		39	1.00	20	
11	0.05	20	II.	40	1.00	20	
12	0.05	20		41	1.00	20	
13	0.05	20		42	1.00	20	V.
14	0.05	10		43	1.00	20	
15	0.05	10		44	2.00	20	
				45	2.00	20	
16	0.05	20		46	2.00	20	
17	0.05	30					
18	0.10	30		47	2.00	20	
19	0.10	30		48	2.00	20	
20	0.10	20		49	2.00	20	VI.
21	0.10	20		50	4.00	20	
22	0.10	20	III.	51	abs. ethanol	20	
23	0.10	20					
24	0.10	20					
25	0.10	20					
26	0.10	20					
27	0.10	20					
28	0.25	20					

number of the experiments presented is limited, it can be seen that metabolism of DHA-4-^{14}C takes place in both types of skin.

The following steroids were isolated from the numerous metabolites detected.



1. *5α-androstane-3,17-dione*

This steroid was isolated from Fraction I. The radioactive substance eluted from the alumina column was purified by partition of the fraction between 90 per cent aqueous ethanol and cyclohexane. The purified extract was chromatographed first on alumina plate, then on paper in the Bush "A" system. The results are shown in Table III. The identity of the radioactive 5α-androstane-3,17-dione was established on the basis of R_f values obtained in two chromatographic systems.

2. *Androst-4-ene-3,17-dione*

This steroid was isolated from Fractions I and II. It will be seen in Fig. 1 that authentic androst-4-ene-3,17-dione-4-^{14}C was present in small quantity in Fraction I of the standard column, but most of the substance was present in

Table II

Distribution of radioactivity on alumina column chromatography of extracts of skin incubated with DHA-4-^{14}C

Incubation 1: Substrate: DHA-4-^{14}C; 10 μc = 570 μg. Specific activity 803 cpm/μg. Incubation in 12 ml KRPG with 10^{-3} M. NAD for 5 hours in O_2

Incubation 2: Substrate: DHA-4-^{14}C; 10 μc = 570 μg. Specific avtivity 1,154 cpm/μg. Incubation in 10 ml KRPG with 10^{-3} M. NAD for 5 hours in O_2

Incubation No.	Flask contents	Tissue incubated mg	Tcpm in extract	Column fractions (see Fig. 1)					
				Fr. I cpm	Fr. II cpm	Fr. III cpm	Fr. IV cpm	Fr. V cpm	Fr. VI cpm
1.	*a)* Pubic skin*	1.205	478.140	4.605	357.000	66.840	15.900	25.365	8.430
	b) Pubic skin	1.180	464.300	5.865	299.940	95.595	24.060	28.139	10.701
2.	*a)* Pubic skin	431	536.432	3.968	72.960	418.740	20.604	13.632	6.528
	b) Abdom. skin	761	557.140	2.020	60.180	458.520	23.280	10.340	4.800
	c) Boiled abdom. skin	481	542.562	150	80.500	410.704	1.140	1.152	1.100
	d) No tissue	—	495.280	720	136.920	352.920	2.340	580	1.800

* With added 10^{-3} M. ATP

Fraction II. Fractions I and II were subjected to preliminary thin-layer chromatography on alumina, the radioactive areas corresponding to authentic androst-4-ene-3,17-dione were eluted, the eluates subjected to the acetic-anhydride-pyridine acetylation procedure of Bush (1961), and rechromatographed on thin-layer alumina-plate. The androst-4-ene-3,17-dione areas from these plates were rechromatographed on thin-layer silica-gel-G plates, and in the Bush "A" paper system. The results are shown in Table IV. The identity of

Table III

Isolation of 5α-androstane-3,17-dione from incubates of pubic skin with DHA-4-^{14}C

Incubation flask (see Table II)	Chromatographic procedures			Tcpm incorporated /g skin
	Alumina column	Alumina-TLC	Paper chromatography	
	Tube No.	R_f	In Bush "A" R_f	
1a) Pubic skin extract	0—5	0.599*	0.754	637
1b) Pubic skin extract	0—5	0.906**	0.754	2.020
Authentic 5α-androstane-3,17-dione	0—5	0.593* 0.881**	0.756	—

* Alumina-TLC: n-hexane-ethyl acetate-glacial acetic acid-abs. ethanol (210 : 30: 2 : 1)
** Alumina-TLC: n-hexane-ethyl acetate-glacial acetic acid-abs. ethanol (180 : 60 : 2 : 1)

Table IV

Isolation of androst-4-ene-3,17-dione from incubates of pubic skin with DHA-4-^{14}C

Incubation flask (see Table II)	Chromatographic procedures				Tcpm incorporated /g skin
	Alumina column	Acetylation			
		TLC		Paper	
	Tube No.	Alumina* R_f	Silica-gel G** R_f	In Bush "A", R_f	
1a) Pubic skin extract	0—15	0.615[1]	0.490	0.497	3.863
2b) Pubic skin extract	0—15	0.463[2]	0.443	0.498	3.991
Authentic androst-4-ene-3,17-dione	4—15	0.609[1] 0,438[2]	0.446	0.499	—

* Alumina-TLC: n-hexane-ethyl acetate-glacial acetic acid-abs. ethanol (180 : 60 : 2 : 1)
** Silica-gel-G—TLC: Benzene-abs. ethanol (92 : 8)

the radioactive androst-4-ene-3,17-dione was established on the basis of R_f values, obtained in the three chromatographic systems.

3. *7-keto-dehydroepiandrosterone (7-keto-DHA)*

This steroid was isolated from Fraction IV. The radioactive substances eluted from the alumina column were chromatographed first on alumina plates, then on paper in the Bush "B_4" system. The radioactive areas corresponding to authentic 7-keto-dehydroepiandrosterone were eluted, the eluates subjected to the acetic-anhydride-pyridine acetylation procedure of Bush (1961) and rechromatographed on paper in the Bush "A" system. The results are shown in Table V. The identity of radioactive 7-keto-DHA and 7-keto-DHA-acetate was established on the basis of R_f values obtained in thin-layer and paper chromatographic systems. These investigations were performed in both cases on female patients, yet it can be seen that the 7-keto-DHA could be isolated only from incubates of No. 1.

4. *7α-Hydroxydehydroepiandrosterone (7α-hydroxy-DHA)*

This steroid was found in Fraction V. The radioactive substances eluted from the alumina column were chromatographed first on alumina plates, then on silica-gel-G in the Lisboa "E" system (1965) and finally on paper in the Bush "B_5" system. The radioactive areas corresponding to authentic 7α-hydroxy-DHA were eluted, the eluates subjected to the acetic-anhydride-pyridine acetylation procedure of Bush (1961). The 7α-hydroxy-DHA-diacetate was rechromatographed on paper in Bush "A" system and/or on alumina plates.

Table V

Isolation of 7-keto-dehydroepiandrosterone (7-k-DHA) from incubates of skin with DHA-4-^{14}C

Incubation flask (see Table II)	Chromatographic procedures				Tcpm incorporated /g skin
	7-k-DHA			7-k-DHA-acetate	
	Alumina column	Alumina-TLC*	Paper chromatography	Paper chromatography	
	Tube No.	Rf	in Bush "B_4" R_f	in Bush "A" R_f	
1a) Pubic skin extract	29—37	0.342[1]	0.640	0.504	1.691
1b) Pubic skin extract	29—37	0.182[2]	0.643	0.502	2.420
2a) Pubic skin extract	29—37	none**	—	—	—
2b) Abdom. skin extract	29—37	none**	—	—	—
Authentic 7-k-DHA	31—35	0.342[1] 0.194[2]	0.639	0.505	—

* Alumina-TLC: n-hexane-ethyl acetate-glacial acetic acid-abs. ethanol (140 : 100 : 0.5 : 2)
** Not detected.

Table VI

Isolation of 7α-hydroxy-dehydroepiandrosterone (7α-OH-DHA) from incubates of skin with DHA-4-^{14}C

Incubation flask (see Table II)	Chromatographic procedures						Tcpm incorporated /g skin
	Alumina column	TLC		Paper chromatography	7α-OH-DHA-diacetate		
		Alumina*	Silica-gel-G**		Paper	TLC	
	Tube No.	R_f	R_f	In Bush "B_5" R_f	In Bush "A" R_f	Alumina*** R_f	
1a) Pubic skin extract	38—46	0.575[1]	0.478[1]	0.453[1]	0.773[1]	—	11.300
1b) Pubic skin extract	38—46	0.344[2]	—	0.406[2]	0.807[2]	0.663[1]	11.140
2a) Pubic skin extract	38—46	0.454[3]	0.429[2]	—	—	0.527[2]	10.468
2b. Abdom. skin extract	38—46	0.491[4]	0.457[2]	—	—	0.636[3]	9.808
Authentic 7α-OH-DHA	43—44	0.578[1]	0.447[1]	0.446[1]	0.771[1]		—
		0.325[2]	0.446[2]	0.401[2]	0.815[2]	0.669[1]	
		0.448[3]				0.527[2]	
		0.491[4]				0.623[3]	

* Alumina-TLC: n-hexane-ethyl acetate-glacial acetic acid-abs. ethanol (20 : 210 : :1 : 10)
** Silica-gel-G-TLC, Lisboa "E" system: ethyl acetate-n-hexane-glacial acetic acid-ethanol (72 : 13.5 : 10 : 4.5)
*** Alumina-TLC: n-hexane-ethyl acetate-glacial acetic acid-abs. ethanol (210 : 30 : : 2 : 1)

The results are shown in Table VI. The identity of radioactive 7α-hydroxy-DHA and 7α-hydroxy-DHA-diacetate was established in thin-layer (alumina and silica-gel-G) and in paper chromatographic systems. Both authentic 7α-hydroxy-DHA and the isolated substance gave the blue spot test with $SbCl_3$ typical of Δ^5-7-hydroxy steroids.

Discussion

It can be seen from Tables I—VI that human skin utilized DHA-4-^{14}C *in vitro*. The principal metabolites were 7α-hydroxy-DHA and androst-4-ene-3,17-dione. However, 7-keto-DHA and 5α-androstane-3,17-dione could be isolated also in smaller quantities from among the numerous metabolites detected (Table VII). No radioactive testosterone could be detected in incubates of human skins.

In vitro C-7-oxygenation and 7α-hydroxylation of DHA has been described in the human adrenal cortex (GALLAGHER 1958, NEVILLE and WEBB 1965, STÁRKA 1965) and in human liver (STÁRKA 1965). 7-keto-DHA was isolated from the urine of patients with normal and raised adrenocortical function (LIEBERMAN et al. 1948). 7α-hydroxy-DHA was found after the administration of DHA in normal man (SCHNEIDER and LEWBART 1959), and even as a normal metabolite of endogenous origin in human urine (STÁRKA and SILINK 1961). The site and mechanism of production of oxygenated dehydroepiandrosterone is an interesting problem. It has been suggested that the hydroxylation and oxygenation of dehydroepiandrosterone occurred in the adrenal gland and in the liver. 7α-hydroxy-DHA and 7-keto-DHA are here reported as conversion products of DHA by *in vitro* incubates of human skin-slices. This observation suggests that skin contains enzyme-systems which *in vitro* are capable of the oxydation of dehydroepiandrosterone *via* the pathway, DHA ⟶ 7α-hydroxy-DHA⟶ 7-keto-DHA.

Table VII

Percentage of conversion of DHA-4-^{14}C to radioactive metabolites in human skin

Incubation flask (see Table II)	5α-androstane-3,17-dione		Androst-4-ene-3,17-dione		7-keto-DHA		7α-hydroxy-DHA	
	Tcpm/g	% conversion	Tcpm/g	% conversion	Tcpm/g	% conversion	Tcpm/g	% conversion
1a) Pubic skin	637	0.133	3.863	0.808	1.691	0.351	11.300	2.363
1b) Pubic skin	2.020	0.435	3.991	0.859	2.420	0.521	11.140	2.399
2a) Pubic skin	—	—	—	—	—	—	10.468	1.951
2b) Abdom. skin	—	—	—	—	—	—	9.808	1.760

The other principal metabolite of DHA isolated from incubates of human skin was androst-4-ene-3,17-dione. It is well known that ovarian, testicular, adrenal and placental tissues contain an enzyme system which catalyzes the oxidation of the Δ^5-3β-hydroxy group of C_{19} steroids to form the Δ^4-3-keto group (SAMUELS et al. 1951). That androst-4-ene-3,17-dione could be isolated from *in vitro* incubates of human skin indicates that human skin contains the Δ^5-3β-hydroxysteroid dehydrogenase system which is capable of the transformation of DHA *in vitro* to androst-4-ene-3,17-dione. Androst-4-ene-3,17-dione is a "basic compound" in the C_{19} series (DORFMAN and UNGAR 1965) and many derivatives can be found in naturally occurring C_{19}-steroids. It is possible that the human skin can also transform this "key compound" according to requirements.

The results of these incubations point to the fact that skin contains enzyme-systems which are capable of utilizing DHA *in vitro*. At present we do not know the significance of these enzymes in human skin. We assume that further study of the utilization of steroids in human skin will furnish more information of skin alterations in endocrine diseases and in the pathogenesis of hirsutism.

Acknowledgement

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ULTRAVIOLETT-ABSORPTIONSSPEKTROPHOTOMETRISCHE UNTERSUCHUNGEN DES LIQUOR CEREBROSPINALIS

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Die Untersuchungen ergaben, daß in der Gestaltung des im Ultraviolett-Bereich gemessenen, charakteristischen Spektrophotogramms (Interferenzkurve) des Liquor cerebrospinalis die Ascorbinsäure eine entscheidende Rolle spielt.

Unter den Methoden der physikochemischen Analyse des Liquor cerebrospinalis scheint in Anbetracht seiner Durchsichtigkeit die UV-Absorptionsspektroskopie eine vielversprechende Möglichkeit zu bieten. Dieses optische Meßverfahren eignet sich zur Bestimmung der Lösungskonzentrationen von Stoffen mit charakteristischer Lichtabsorption. Im Falle von Substanzgemischen dagegen ist die Auswertung des Spektrogramms nicht einfach, weil die Absorptionsmaxima der einzelnen Komponenten einander überlagern und eine Interferenzkurve resultieren kann. Dies ist auch bei den Liquoruntersuchungen der Fall.

Mit der Spektrophotometrie des Liquors hat sich als erster Shionoya [1] befaßt. Er fand bei pathologischen Lumbalflüssigkeiten andere Absorptionswerte als bei normalen und konnte einen Zusammenhang zwischen Kurvenhöhe und Eiweißgehalt des Liquors nachweisen. Veraguth und Opitz [2] kamen im wesentlichen zu analogen Feststellungen, indem bei organisch-neurologischen Krankheiten der zuvor enteiweißte Liquor höhere Absorptionswerte zeigte. Auch Damjanovich und Mitarbeiter [3] sowie Jacobi und Mitarb. [4, 5] wiesen nach, daß verschiedene menschliche Lumbalflüssigkeiten spektrographisch weitgehend voneinander abweichen. Nach den Untersuchungen von Karczag und Hanák [6] sind für die Abweichungen im Spektrogramm der menschlichen Liquore vor allem Proteine und Harnsäure verantwortlich. Plaut und Mitarb. [7] haben ihre mit 2,6-Dichlorphenolindophenol vorgenommenen chemischen Bestimmungen des Vitamin C-Gehaltes im Liquor auf spektrophotometrischem Wege nachgeprüft.

Mit der Bewertung der Liquor-Spektrogramme haben sich M. Spiegel-Adolf und Mitarbeiter [8—30] eingehend befaßt. Bei verschiedenen pathologischen Fällen untersuchten sie die Liquor-Spektrogramme und konnten Korre-

lationen zwischen der Krankheit und der Wellenlänge und Höhe der Absorptionsmaxima feststellen. So fanden sie, daß bei Schizophrenie, Gehirnerschütterung, Paralysis progressiva, metastatischen und primären Gehirntumoren, Epilepsie und bei mit konvulsiven Symptomen einhergehenden Erkrankungen des Zentralnervensystems das Spektrogramm der Lumbalflüssigkeit sich in seinen — bei gleicher Wellenlänge gemessenen — Extinktionswerten weitgehend von den normalen unterscheidet.

Die sich mit der Liquor-UV-Spektrophotometrie beschäftigenden Autoren stimmen darin überein, daß in dem Zustandekommen des typischen S-förmigen Spektrogramms der Lumbalflüssigkeit verschiedene auch physiologisch vorkommende Stoffe eine Rolle spielen, unter denen die normalen und pathologischen Proteine, die Nukleinsäuren und ihre Derivate, sowie Harnsäure und Ascorbinsäure die größte Beachtung verdienen; in der Frage aber, welche von den im Liquor chemisch nachweisbaren Substanzen in der Gestaltung des Absorptionsspektrums die wichtigsten sind, gehen die Meinungen auseinander. SPIEGEL-ADOLF und Mitarb., sowie auch NODA [31], HACK [32] und STRAIT [33] vermuten eine eventuelle Rolle der Ascorbinsäure; doch sprechen SPIEGEL-ADOLF und Mitarb. auf Grund ihrer parallel mit der Spektrophotometrie durchgeführten chemischen Bestimmungen die Hauptrolle den Nukleinsäuren und ihren Abkömmlingen, sowie den Proteinen, bzw. den pathologischen Proteinen zu. Auch die Rolle der Ascorbinsäure ist ihres Erachtens nicht zu vernachlässigen, deshalb konvertieren sie deren mittels chemischer Bestimmung erhaltene Werte zu Absorptionswerten und ziehen sie von der Gesamtabsorption ab.

Die Liquor-UV-Spektrophotometrie hat eine sehr umfangreiche Literatur, dennoch hat sich bis auf den heutigen Tag kein einheitlicher Standpunkt betreffs der Frage herausbilden können, welcher Stoff bei der Spektrophotometrie des Liquors eigentlich gemessen wird und welchen Platz die Liquor-Spektrophotometrie in der Liquordiagnostik einnimmt.

Das Ziel unserer Untersuchungen war zu ermitteln,

1. welchem Stoff in der Gestaltung des Liquor-Spektrogramms eine dominante Bedeutung zukommt,

2. wodurch die Abweichungen in den einzelnen Liquor-Spektrogrammen bedingt sind.

Untersuchungsmaterial und Methoden

Es wurden die Spektrogramme des mittels Lumbalpunktion von 50 Personen gewonnenen Liquors aufgenommen.

Unser Krankengut bestand aus 18 Patienten mit Kopfschmerzen, 8 mit Urämie und chronischer Glomerulonephritis, 7 mit Epilepsie, 12 mit hypertensiver Encephalopathie und 5 Fälle mit positivem EEG-Befund ohne organisch-neurologische Abweichungen.

Da der Verlauf des Spektrogramms keinerlei Zusammenhang mit der Diagnose der Kranken aufwies, wurde in Tabelle I von der Anführung der einzelnen Diagnosen abgesehen. Zur Spektrometrie fanden lediglich vollkommen klare Liquorproben Verwendung. Da die abgelassene Lumbalflüssigkeit fallweise nur 5—6 ml betrug und die Extinktionswerte der nativen

Liquore sich als zu hoch erwiesen, haben wir im ersten Teil unserer Untersuchungen die Liquorproben mit bidestilliertem Wasser im Verhältnis 1 : 5 verdünnt.

Die optischen Messungen erfolgten größtenteils mit Hilfe des *Spektromom 201* (MOM, Budapest), und zum kleineren Teil mit den Apparaten *Unicam (Sp 500)*, *Hilger* (UVISPEK H 700) und *Beckmann DU* in Quarzküvetten von 10 mm Schichtdicke. Mit den einzelnen Geräten wurden weder hinsichtlich der Wellenlänge, noch der Extinktionswerte nennenswerte Unterschiede erhalten (in der Wellenlänge ergaben sich Abweichungen von höchstens 1 mμ, und in den Extinktionswerten bis zu 0,010). Extinktion und Wellenlänge sind am Koordinatensystem dargestellt.

Andere Autoren [34] haben den Transmissionsprozentsatz gegenüber der Wellenlänge dargestellt. Die zweierlei Darstellungsmethoden können Anlaß zu Mißverständnissen geben, da nach dem System von DELBA die Minima des Spektrogramms die Absorptionsmaxima bedeuten.

Der Meßbereich lag zwischen 210—300 mμ. Anstatt der schon erwähnten Verdünnung mit dest. Wasser haben wir den Liquor im weiteren — aus später zu erörternden Gründen — mit 0,01 m/l NaCN-Lösung 1 : 5 verdünnt.

In sämtlichen Liquorproben wurden außer dem Spektrogramm auch der Eiweißgehalt (mit der Sulfosalicylsäuremethode von UJSÁGHY) und der Ascorbinsäuregehalt (nach ROE und KUETHER) untersucht.

Ergebnisse

Die Spektrogramme der Lumbalflüssigkeit stellen typische, horizontal verlaufende S-förmige Kurven dar, deren absteigender Schenkel das Minimum bei 240—245 mμ erreicht, bei 245—265 mμ wieder ansteigt, bei 265—268 mμ

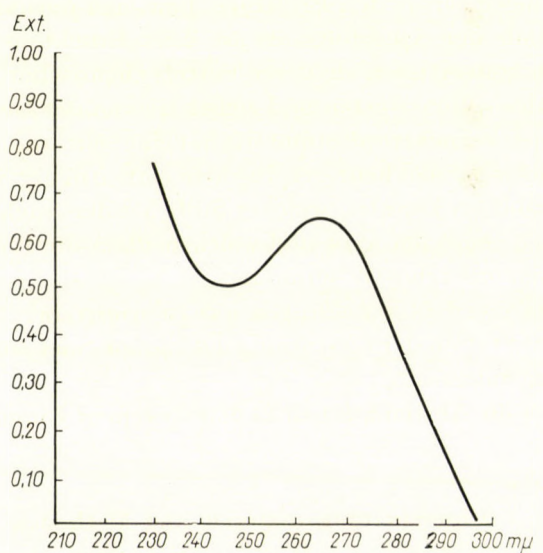

Abb. 1. Spektrogramm einer wässerigen Liquorverdünnung 1 : 5, gemessen innerhalb von 30 Minuten nach der Liquorentnahme

ein charakteristisches Maximum zeigt und anschließend allmählich auf 300 mμ zurückgeht. Die typische Kurvenform des Liquors veranschaulicht Abb. 1.

Wie Abb. 2 zu entnehmen ist, wichen die Spektren der einzelnen Liquore betreffs ihrer bei der gleichen Wellenlänge gemessenen Extinktionswerte stark

Tabelle I

Fall	Protein mg%	Ascorbinsäure mg %	Ext.—Max. bei 264—270 mμ
1. N. J. ♂	46		0,202
2. T. J. ♂	34	2,65	0,700
3. P. I. ♂	16		0,367
4. C. B. ♂	40		0,189
5. T. D. ♂	11		0,403
6. P. M. ♂	35		0,168
7. B. J. ♂	21		0,252
8. T. Gy. ♀	34		0,532
9. Cs. Gy. ♀	82*		0,529
10. L. I. ♂	46	0,45	0,385
11. R. L. ♀	41	1,0	0,640
12. E. K. ♀	12	0,4	0,564
13. P. I. ♀	1	3,3	0,643
14. P. S. ♂	20		0,868
15. F. H. ♂	36	3,2	0,648
16. V. ♀	20	3,0	0,496
17. D. J. ♂	18	3,3	0,615
18. O. N. ♂	36	1,8	0,605
19. B. A. ♀	33	3,0	0,710
20. J. G. ♂	14	3,0	0,718
21. Sz. Gy. ♀	41	1,5	0,352
22. K. J. ♀	32	3,0	0,740
23. Ny. E. ♀	45	1,6	0,418
24. N. J. ♂	75*	0,7	0,419
25. P. S. ♂	22	1,7	0,569
26. S. ♀	29	1,9	0,567
27. S. M. ♂	30	1,0	0,880
28. Ny. F. ♀	31	0,6	0,638
29. K. E. ♂	10	1,0	0,592
30. M. B. ♀	17	2,5	0,494
31. R. J. ♂	17	4,0	0,700
32. Sz. I. ♂	20	2,7	0,418
33. L. O. ♂	15	3,2	0,619
34. H. I. ♂	30	1,8	0,687
35. T. J. ♀	21	3,6	0,682

* Der Liquor war blutig.
Die Verdünnung der Liquorproben 1—17 geschah mit dest. Wasser.
* Der Liquor war blutig.
Die Verdünnung der Liquorproben 18—35 geschah mit 0,01 M NaCN.

Tabelle I (Fortsetzung)

Fall	Protein mg %	Ascorbinsäure mg %	Ext.—Max. bei 264—270 mμ
36. M. I. ♂	23	3,5	0,632
37. P. É. ♂	26	3,0	0,600
38. K. I. ♂	36	1,7	0,438
39. M. ♀		0,5	0,446
40. Cs. M. ♂	23	3,4	0,622
41. M. L. ♂	20	2,6	0,615
42. T. J. ♀	23	2,1	0,540
43. O. I. ♂	30	4,0	0,675
44. V. J. ♀	25	1,8	0,640
45. P. Gy. ♂	27	3,5	0,553
46. M. J. ♂	30	5,0	0,800
47. S. F. ♂	18	1,2	0,264
48. B. L. ♀	25	3,0	0,638
49. K. K. ♀	13	3,8	0,790
50. Sz. M. ♂	16	3,7	0,698

Die Verdünnung der Liquorproben 36—50 geschah mit 0,01 M NaCN.

ab. Der besseren Übersichtlichkeit halber haben wir nur die Kurven einiger Liquore dargestellt.

In Tabelle I sind der Eiweiß- und Ascorbinsäuregehalt sowie die Wellenlängen und die Extinktionshöchstwerte aller untersuchten Lumbalflüssigkeiten zusammenfassend dargestellt.

Auffallend war, daß die binnen 30 Minuten nach der Abnahme derselben Liquore gemessenen Extinktionswerte nicht mit den später gemessenen übereinstimmten; die letzteren fielen — der Inkubationsdauer proportional — konsequent niedriger aus. Am augenfälligsten war das Nachlassen der Extinktionswerte im Bereich des Absorptionsmaximums (Abb. 2, 3 und 4).

Im Schrifttum konnten keine Angaben betreffs einer Abnahme der Extinktionswerte des Liquor-Spektrogramms mit der Zeit gefunden werden. Wir nahmen an, daß in der wässerigen Verdünnung die Konzentration eines an der Luft sich zersetzenden oder umgewandelten Stoffes abnimmt. Hierbei trachteten wir eine Antwort auf die Frage zu erhalten, welcher Stoff bei der definitiven Gestaltung der Interferenzkurve des Liquors ausschlaggebend ist.

In Abb. 5, 6 und 7, sowie in Tab. II sind die Spektrogramme, die Absorptionsmaxima und die einschlägigen Literaturangaben betreffs der wichtigen Stoffe, deren Absorptionsmaxima zwischen 250 und 290 mμ liegen, dargestellt. Die Abbildungen und Tabellen wurden nach OTTING [35] angefertigt.

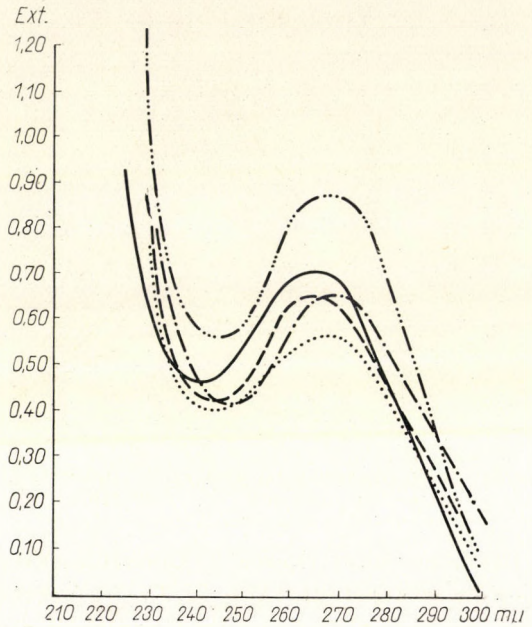

Abb. 2. Spektrogramme wässeriger Liquorverdünnungen 1 : 5, gemessen innerhalb von 30 Minuten nach der Liquorentnahme. (—···— Tabelle I., Liquor Nr. 14; —·— Tabelle I., Liquor Nr. 13; - - - Tabelle I., Liquor Nr. 15; .... Tabelle I., Liquor Nr. 12 und — Tabelle I., Liquor Nr. 2)

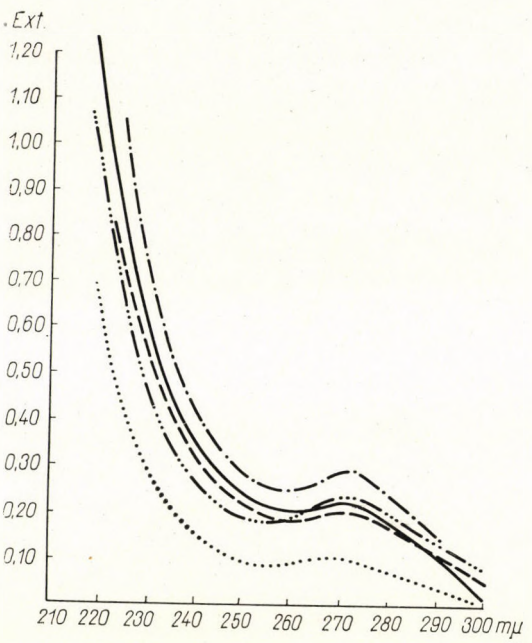

Abb. 3. Spektrogramme der gleichen Liquorproben, 8 Stunden nach der Liquorentnahme, (Bezeichnungen wie in Abb. 2.)

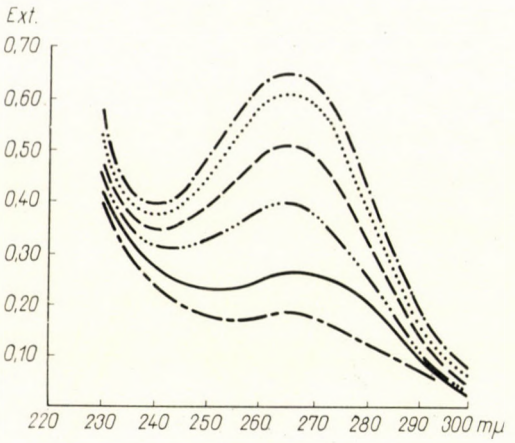

Abb. 4. Veränderung des Spektrogramms der wässerigen Liquorverdünnung —·—·— sofort .... 30, - - - - 60, —···— 120,—240—360 und - — - — 480 Minuten nach der Liquorentnahme

Nach dem Passieren einer Dowex 2 Anionenaustauscher-Säule nehmen die Extinktionswerte wesentlich ab (Abb. 8). Dieser Versuch beweist lediglich, ob es sich bei dem obenerwähnten, in der Gestaltung der charakteristischen

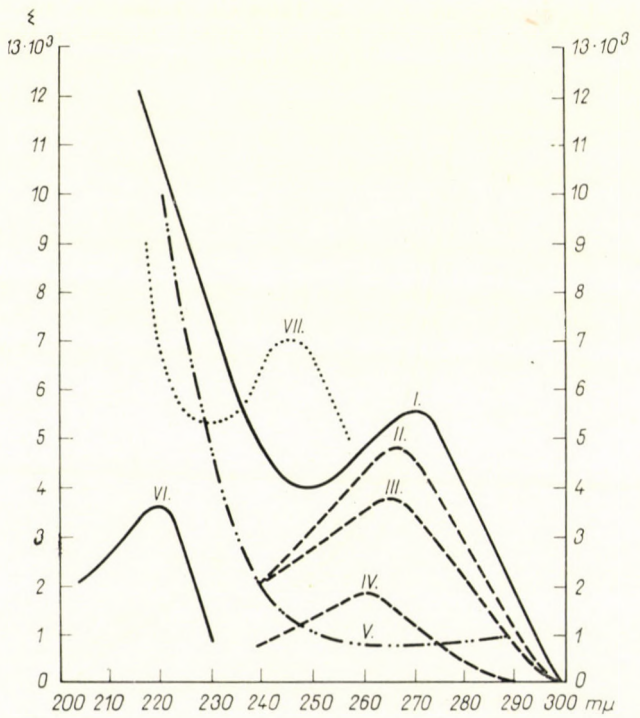

Abb. 5. I. 5-Oxymethylcytosin, II. Flavinadenindinukleotid, III. Lactoflavin, IV. Adenosin-5-phosphat, V. Urea, VI. Tryptophan, VII. Barbiturat

Tabelle II

Abbildung	Substanz	Ext.—Max. mμ	pH	Schrifttum
5./I.	5-Oxymethylcytosin	270	7,4	*Wyatt, G. R., Cohen, S. S.:* Biochem. J. **55,** 776 (1953).
5./II.	Flavinadenindinukleotid	265	—	*Whitby, L. G.:* Biochem. J. **54,** 440 (1953).
5./III.	Lactoflavin	265	—	*Whitby, L. G.:* Biochem. J. **54,** 440 (1953).
5./IV.	Adenosin-5-phosphat	262	—	*Whitby, L. G.:* Biochem. J. **54,** 440 (1953).
5./V.	Urea	—	—	*Klotr, I. M., Askonnis, Th.:* J. Amer. chem. Soc. **69,** 803 (1947).
5./VI.	Tryptophan	220	6—8	*Saidel, L. J., Goldfarb, A. R., Waldman, S.:* J. biol. Chem. **197,** 287 (1952).
5./VII.	Barbitursäure	245	—	*Klotr, I. M., Askonnis, Th.:* J. Amer. chem. Soc. **69,** 803 (1947).
6./I.	Desoxyribonukleinsäure	260	—	*Shlenk, F.:* Advanc. Enzymol. **9,** 486 (1949).
6./II.	Glykolsäureoxydase	275	—	*Zelitch, I., Ochoa, S.:* J. biol. Chem. **201,** 712 (1953).
6./III.	Harnsäure	290	6,8	*Schlenk, F.:* Advanc. Enzymol. **9,** 481 (1949).
6./IV.	Guanin	250	6,8	*Schlenk, F.:* Advanc. Enzymol. **9,** 481 (1949).
6./V.	Adenosintriphosphat	258	—	*Schlenk, F.:* Advanc. Enzymol. **9,** 488 (1949).
6./VI.	Nikotinamid	265	7,0	*Cantoni, G. L.:* J. biol. Chem. **189,** 212 (1951).
6./VII.	Lactoflavinphosphat	265	7,4	*Zelitch, I., Ochoa, S.:* J. biol. Chem. **201,** 712 (1953).
7./I.	Uridin	262	7,2	*Fox, J. J., Shugar, D.:* Biochim. biophys. Acta (Amst.) **9,** 379 (1952).
7./II.	Thymidin	267	7,2	*Fox, J. J., Shugar, D.:* Biochim. biophys. Acta (Amst.) **9,** 381 (1952).
7./III.	Cytidin	272	7,2	*Fox, J. J., Shugar, D.:* Biochim. biophys. Acta (Amst.) **9,** 372 (1952).
7./IV.	Uracyl	258	7,2	*Shugar, D., Fox, J. J.:* Biochim. biophys. Acta (Amst.) **9,** 205 (1952).
7./V.	Thymin	265	7,2	*Shugar, D., Fox, J. J.:* Biochim. biophys. Acta (Amst.) **9,** 208 (1952).
7./VI.	Orotsäure	278	7,2	*Shugar, D., Fox. J. J.:* Biochim. biophys. Acta (Amst.) **9,** 211 (1952).
7./VII.	Cytosin	268	7,2	*Shugar, D., Fox, J. J.:* Biochim. biophys. Acta (Amst.) **9,** 202 (1952).
7./VIII.	5-Methylcytosin	275	7,2	*Shugar, D., Fox, J. J.:* Biochim. biophys. Acta (Amst.) **9,** 204 (1952).

Absorptionsmaxima eine entscheidende Rolle spielenden Stoff um eine anionische und nicht basische Substanz handelt, wie z. B. Cytosin, 5-Oxymethylcytosin, Uridin, Uracyl, Thymin, Thymidin, Cytidin, die in Anbetracht ihres um 265 mμ gelegenen Extinktionsmaximums in Frage kamen.

Die in der Lumbalflüssigkeit enthaltene [36, 47] und in pathologischen Zuständen erhöhte [37] Brenztraubensäure wurde auch in Betracht gezogen,

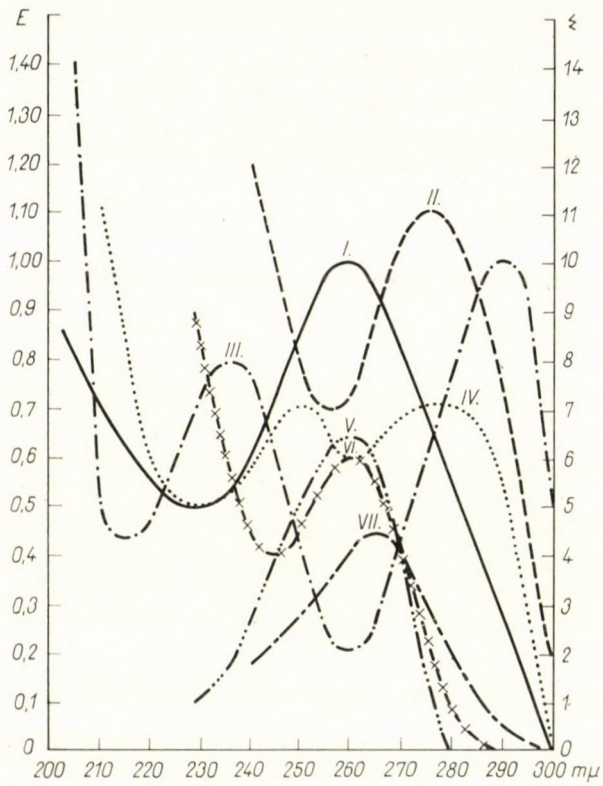

Abb. 6. I. Desoxyribonukleinsäure, II. Glykolsäureoxydase, III. Harnsäure, IV. Guanin, V. Adenosintriphosphat, VI. Nikotinamid, VII. Lactoflavinphosphat

da das Absorptionsmaximum ihrer wässerigen Lösung laut eigenen Messungen ebenfalls bei 265 mμ liegt. Gegen eine entscheidende Rolle der Brenztraubensäure spricht allerdings der Umstand, daß die Extinktionswerte ihrer wässerigen Lösung weder beim Stehen, noch bei 30 Minuten langer Luftdurchströmung wesentlich geringer werden, und ferner, daß die molekuläre Extinktion der Ascorbinsäure erheblich größer ist als die der Brenztraubensäure (Abb. 9 und 14).

Nach den eingangs zitierten Autoren wird die Form bzw. der Verlauf des Liquor-Spektrogramms außer von den Eiweißzerfallprodukten und Nukleinsäurederivaten auch vom Eiweiß- und Harnsäuregehalt des Liquors beeinflußt.

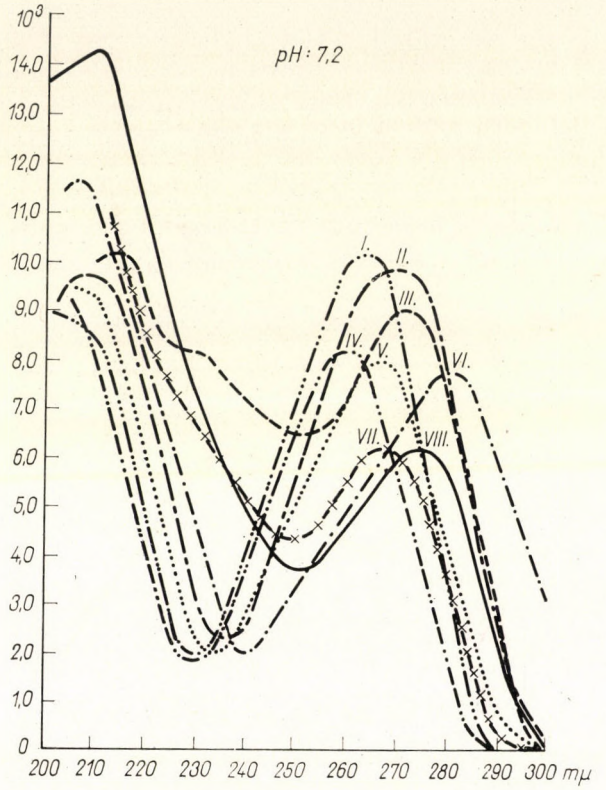

Abb. 7. I. Uridin, II. Thymidin, III. Cytidin, IV. Uracyl, V. Thymin, VI. Orotsäure, VII. Cytosin, VIII. 5-Methylcytosin

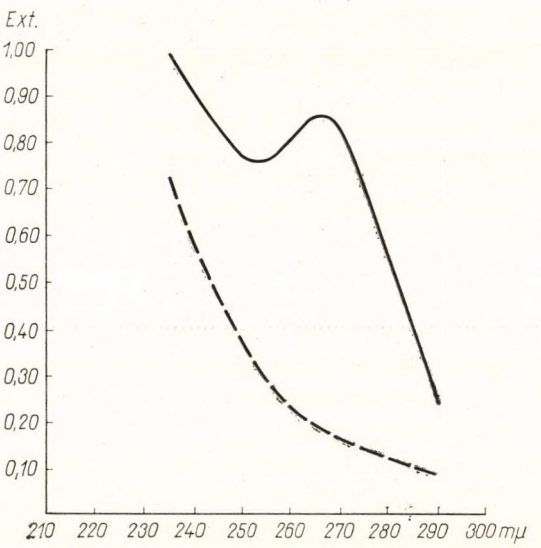

Abb. 8. Spektrogramm einer mit 0,01 M/1 NaCN hergestellten Liquorverdünnung 1 : 5 — vor und - - - nach dem Passieren einer Dowex 2 Anionen-Austauschersäule (Tabelle I., Liquor Nr. 27.)

Das Eiweiß, als UV-absorptionsfähiges Material mit einer Bande bei 280 mμ [39, 40], kann natürlich in der Wellenlängenveränderung eine Rolle spielen. Nach eigenen Befunden werden in 20 mg% Se-Eiweiß enthaltenen Ascorbinsäure-Verdünnungsreihen die Form der Spektrogramme und die Absorptionshöchstwerte in der Nähe von 265 mμ in erster Linie durch den Ascorbinsäuregehalt der Mischlösungen beeinflußt (Abb. 10 und 11); nahe der Wellenlänge von 290 mμ ist — besonders bei Hyperuricaemie — auch die Harnsäure von Be-

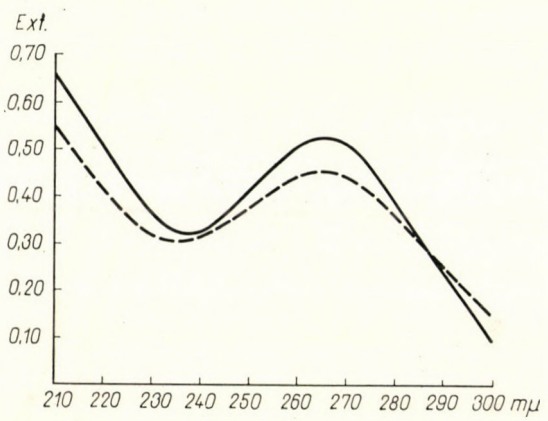

Abb. 9. 3 mg%ige (340 Mikromol) l Brenztraubensäurelösung (in Tyrodelösung) — vor und - - - nach 30 Minuten langer Durchströmung mit Luft

deutung [41—44]. Das Spektrogramm dieser Stoffe verringert sich jedoch weder beim Stehen an der Luft noch unter dem Einfluß von O_2 — oder Luftdurchströmung.

30 Minuten langes Durchperlen mit Luft oder 1:100 verdünntes H_2O_2 bedingt ähnliche Veränderungen im Spektrogramm, wie ein längeres Stehen an der Luft.

Bei der Verdünnung der Lumbalflüssigkeit mit 0,01 M/l NaCN-Lösung im Verhältnis 1:5 kommt die Verringerung der Extinktionswerte weder auf Durchströmung mit Luft, noch auf Inkubation bei Raumtemperatur zustande (Abb. 12).

Die zur Verdünnung benutzte CN-Lösung wurde nach HERBERT [44] und PIOAN [45] angewandt, um die die Zersetzung der Ascorbinsäure katalysierenden Schwermetallsalze zu binden.

Auf die Ascorbinsäure wurde unsere Aufmerksamkeit dadurch gelenkt, daß nach Auftropfen einiger Liquorproben auf zuvor belichtetes Photopapier an der Auftropfstelle ein schwarzbräunlicher Fleck erschien. Einen Fleck gleicher Intensität vermochte auch 3 mg%ige wässerige Ascorbinsäurelösung hervorzurufen.

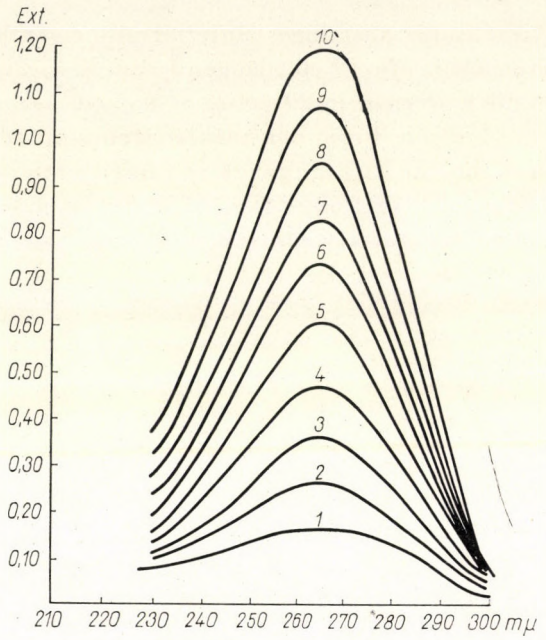

Abb. 10. Spektrogramme von 1—10 mg%iger Ascorbinsäurelösung. (Verdünnung 1 : 5 mit 0,01 M/1 NaCN. Endkonzentration: 0,2—2,0 mg%.)

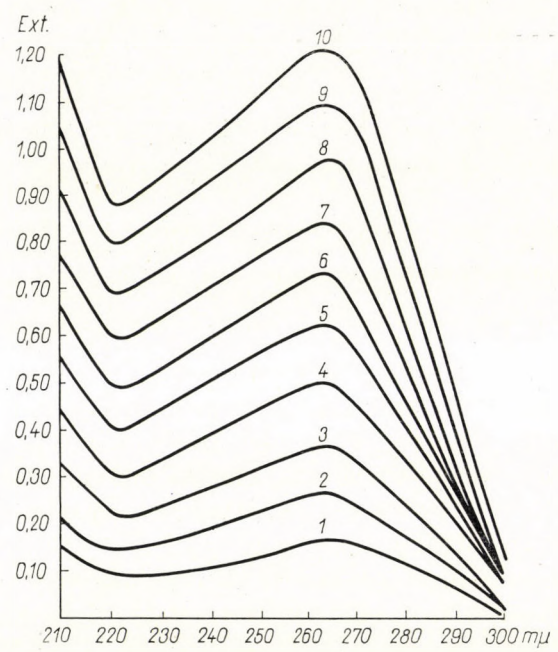

Abb. 11. Spektrogramme von 1—10 mg%iger Ascorbinsäurelösung (in 20 mg%iger Eiweißlösung). (Verdünnung 1 : 5 mit 0,01 M/1 NaCN, Endkonzentration für Ascorbinsäure: 0,2—2,0 mg%, für Eiweiß: 4 mg%.)

Die Konzentration der Ascorbinsäure in der Lumbalflüssigkeit liegt zwischen 0,3—4,18 mg% [45, 46, 47, 48, 49] und ihr Absorptionsmaximum bei 265 mμ [32, 34, 44, 50 und eigene Befunde].

Sauerstoff- und Luftdurchströmung, sowie Stehen an der Luft und Oxydationsmittel vermögen das Spektrogramm der Ascorbinsäure zu beeinflussen, da

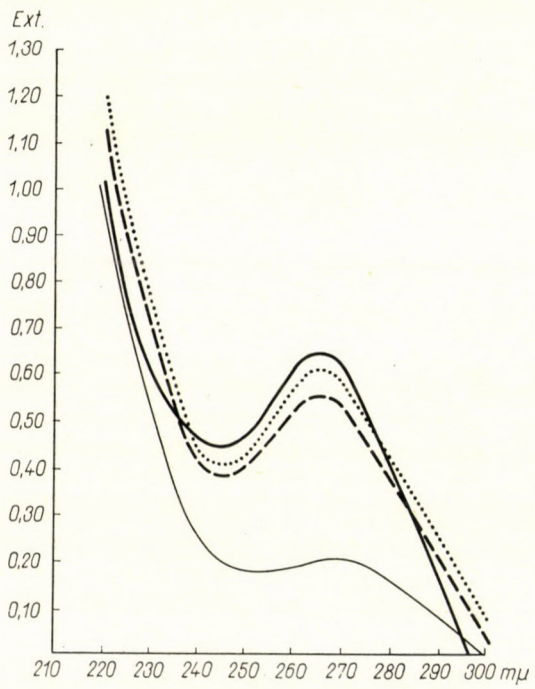

Abb. 12. Spektrogramm einer wässerigen Liquorverdünnung 1 : 5 (Tabelle I., Liquor Nr. 18) — 20 und — 480 Minuten nach der Liquorentnahme. Spektrogramm eines ebenso mit 0,01 M/l NaCN verdünnten Liquors (Tabelle I., Liquor Nr. 13) ..... 20 und - - - - 480 Minuten nach der Entnahme

diese in spezifischer Lichtabsorption nicht mehr fähige Dehydroascorbinsäure umgewandelt wird (Abb. 13). Eine 10^{-3} Mol/l NaCN-Lösung schützt nach HERBERT [44] und PIOAN [45] die Ascorbinsäurelösung vor Oxydierung. Die NaCN-Lösung gibt eine — Wasser gegenüber zu vernachlässigende — geringgradige Extinktion (0,015).

Der Zusammenhang zwischen Extinktionsmaxima der verschiedenen Liquore und dem nach ROE und KUETHER gemessenen Ascorbinsäuregehalt wurde mathematisch erwiesen.

33 Messungspaare wurden analysiert. Die Vitamin C-Werte sind mit x und die Extinktionswerte mit y bezeichnet. Der Korrelationskoeffizient der beiden

Daten wurde auf Grund der Gleichung:

$$r = \frac{\sum_{i=1}^{33} (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{33} (x_i - \bar{x})^2 \sum_{i=1}^{33} (y_i - \bar{y})^2}} = 0{,}685$$

berechnet, wobei der Korrelationskoeffizient r, und $\bar{x}$ und $\bar{y}$ den entsprechenden mathematischen Mittelwert bedeuten $\begin{pmatrix} \bar{x} = 2{,}47 \\ \bar{y} = 0{,}599 \end{pmatrix}$.

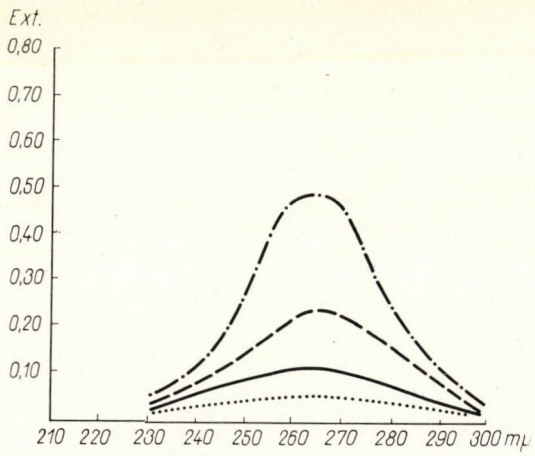

Abb. 13. Spektrogramm einer 1 mg%igen wässerigen Ascorbinsäurelösung. Gemessen: —·— sofort, - - - nach 30, — 60 und .... 120 Minuten langem Stehen bei 20 °C

Bezeichnend für den Zusammenhang ist, wie weit r 1 nahekommt.

Somit war also zwischen den untersuchten Parametern eine enge Korrelation und zwar ein gerades Verhältnis (der Wert von r ist positiv) festzustellen. Die Streuung des Korrelationskoeffizienten war

$$s_r = \frac{1 - r^2}{\sqrt{n - 1}} = 0{,}094$$

Wir haben den Wert von r der Signifikanzprobe unterworfen. Da dieser nicht von normaler Verteilung ist, bedienten wir uns der Formel:

$$t_{n-2} = \frac{r}{\sqrt{1 - r^2}} x\ n - 2 = 5{,}23$$

daher $p < 0{,}1\%$.

Die Signifikanz haben wir auch auf Grund der FISCHERschen Transformation erwiesen:

$$Z = 0{,}839. \text{ Streuung von } Z = 0{,}182$$

Für die Durchführung der mathematischen Berechnungen sei Herrn A. TÖRÖK auch an dieser Stelle unser Dank angesprochen.

Besprechung

Es wurde festgestellt, daß im Zustandekommen der Absorptionsspektrum-Kurven der Lumbalflüssigkeit der Ascorbinsäuregehalt der wesentlichste Faktor ist. Unsere Befunde stimmen mit den Feststellungen von SPIEGEL-ADOLF [13, 14], NODA [31], HACK [32] und STRAIT [33] überein — mit dem Unterschied, daß unseres Erachtens das Vitamin C den wichtigsten, und die übrigen Stoffe (Eiweiß, Harnsäure, Nukleinsäuren und ihre Derivate) nur akzidentelle Faktoren darstellen.

In unseren Versuchen kam — obzwar der Zusammenhang zwischen Vitamin C mg%-Gehalt und Extinktionswerten mathematisch bewiesen werden konnte ($r = 0{,}685$) — der Extinktionskoeffizient dem 1,00-Wert doch nicht ganz nahe. Die Ursache hierfür ist in mehreren Faktoren zu suchen. Nach HIRST [51] und WORTIS [46] liegt im Organismus ein Teil der Ascorbinsäure in der Dehydro-Form vor, die schon keine selektive Absorption mehr besitzt, deren biologische Wirkung jedoch noch erhalten ist.

Dies ist ein überaus wichtiger Umstand, da in der Zelloxydation das Redoxsystem der reduzierten und oxydierten Ascorbinsäure eine wichtige Rolle einnimmt. Spektrophotometrisch ist nur der Gehalt an reduzierter Ascorbinsäure erfaßbar.

Bei Anwendung der 2,6-Dinitrophenylhydrazin-Methode wird die Ascorbinsäure mit Oxydationsmitteln in Dehydroascorbinsäure umgewandelt und letztere als Osazon bestimmt. Die von PLAUT und Mitarbeitern [7] spektrophotometrisch erhaltenen Vitamin C-Werte stehen mit den mit Hilfe der Dichlorphenolindophenol-Methode gefundenen gut im Einklang. Dies erklärt sich daraus, daß die Autoren vor der spektrophotometrischen Messung die Vitamin C-haltige, wässerige Lösung mit H_2S durchströmt, und dadurch die Dehydroascorbinsäure in Ascorbinsäure zurückverwandelt hatten. (Die H_2S-Durchströmung schützt gleichzeitig die Ascorbinsäure vor dem oxydierenden Einfluß der Luft.)

Die andere Ursache ist, daß das Spektrogramm offenbar auch durch andere Stoffe des Liquors — wenn auch nicht definitiv — beeinflußt wird.

Im folgenden seien jene Argumente angeführt, welche — direkt oder indirekt — unsere Vermutung unterstützen, wonach nämlich in der Gestaltung

des Absorptionsmaximums der Lumbalflüssigkeit die Ascorbinsäure die entscheidende Rolle spielt:

Liquor und Ascorbinsäure haben

1. ihr Absorptionsmaximum gleichermaßen bei 265 mμ,

2. die Extinktionswerte ihrer wässerigen Verdünnungen werden nach dem Stehen geringer,

3. nach 30 Minuten Stehen an der Luft bzw. O_2-Durchströmung kommt die Verringerung der Extinktionswerte ebenso zustande, was — wegen ihrer höchst selektiven Absorption — weder den Nukleinsäuren und ihren Abkömmlingen, noch dem Protein oder der Harnsäure zuzuschreiben ist, da ihre Absorptionsmaxima bei 280 bzw. 290 mμ liegen und das Spektrogramm während des Stehens keine Verminderung erfährt.

4. Eine mittelbare Stütze für unsere Behauptung ist die Mitteilung von CAZZULLO [52, 53], wonach die Liquorproben, wenn sie nach der Entnahme $^1/_2$—23 Stunden bei Zimmertemperatur stehen, eine 32—100%ige Verminderung ihres chemisch ermittelten Ascorbinsäuregehaltes erfahren.

5. Das CN-haltige Verdünnungsmittel verhindert die Extinktionsverminderung,

6. nach Passieren einer Anionen-Austauschersäule liegt das Extinktionsmaximum des Liquors wesentlich niedriger,

7. der Verlauf der Liquorkurven wird zwar bei 280 mμ auch durch die Eiweißkonzentration beeinflußt, für die Absorption bei 265 mμ ist jedoch die Ascorbinsäure verantwortlich.

Einen Zusammenhang zwischen der Diagnose unserer Fälle und den Spektrogrammen konnte nicht nachgewiesen werden. Die Analyse der Liquor-Spektrogramme und die Sammlung der daraus resultierenden Erfahrungen können zweifelsohne in allen jenen Fällen nützlich sein, wo der klinische Verdacht auf eine Läsion des Zentralnervensystems besteht, die Ergebnisse der Routineuntersuchungen des Liquors aber nicht von den normalen Werten abweichen.

Die Auswertung der Liquor-Spektrogramme ist keine abgeschlossene Frage. Der Gehalt an reduzierter Ascorbinsäure ist spektrometrisch meßbar, und weitere Untersuchungen sind berufen die Frage zu klären, ob das Verhältnis der Komponenten des im Liquor enthaltenen Ascorbinsäure—Dehydroascorbinsäure-Redoxsystems unter pathologischen Umständen verändert ist.

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РЕЗЮМЕ

ВОПРОСЫ ОБМЕНА ЖЕЛЕЗА И АНЕМИИ ПРИ СИСТЕМНОЙ КРАСНОЙ ВОЛЧАНКЕ И ПРИ РЕВМАТОИДНОМ АРТРИТЕ

Т. БУРГЕР, Д. БРАШ и Б. КЕСТХЕИ

Авторы выявили значительное повышение обмена железа при системной красной волчанке и при ревматоидном артрите. Однако встраивание радиожелеза в эритроциты у 7 из 11 больных с системной красной волчанкой и у 7 больных с ревматоидным артритом было понижено. Результаты измерений, проведенных in vivo, указывают на повышенное депонирование железа в печени и в селезенке, и можно предполагать, что это соединение железа не дает реакции с берлинской лазурью.

На основании литературных данных и собственных исследований авторы считают, что встречающаяся при этих двух болезнях анемия вызывается тремя факторами: пониженной активностью костного мозга, сокращением продолжительности жизни эритроцитов и умеренным недостатком в железе.

ПРОБЛЕМЫ ОПРЕДЕЛЕНИЯ ПРОДОЛЖИТЕЛЬНОСТИ ЖИЗНИ ТРОМБОЦИТОВ

Т. БУРГЕР

Автор излагает свой метод определения продолжительности жизни тромбоцитов при помощи изотопа Cr^{51}. Эффективность изотопа прямо пропорциональна его активности и густоте взвеси тромбоцитов. Оптимальная температура: 18—22° С. Определяя ежедневно во взятых обратно пробах крови активность тромбоцитов, наблюдается экспоненциальное понижение активности тромбоцитов и время полураспада тромбоцитов 3,5—4,5 дней. Полную продолжительность жизни тромбоцитов автор определил в 9—11 дней как в отношении гомологичных, так и автологичных тромбоцитов, независимо от того, применял ли он совместимую по групповой принадлежности АВО, или несовместимую кровь. По мнению автора, истинная продолжительность жизни тромбоцитов, вероятно, более длительная.

Место гибели тромбоцитов еще не установлено точно, но на основании измерений, проведенных in vivo, при этом роль играют печень, селезенка и в меньшей мере легкие.

Автор подчеркивает, что метод пригоден также и для клинических исследований и может оказывать большую помощь при дифференциальной диагностике геморрагических диатезов.

ДАННЫЕ К ПАТОГЕНЕЗУ ОТЕКА ЛЕГКИХ

Д. КЕВЕР, Е. СЕЧ и М. ЗОМБОРИ

Авторы создали у крыс экспериментальную гиперволемию введением 2,5%-ого раствора глюкозы, 0,9%-ого раствора поваренной соли или 10%-ого раствора глюкозы, в количестве, соответствующем 3%-ам веса тела животных. При одновременном введении в хвостовую вену 50 мЕ питрессина и через полчаса еще 50 мЕ питрессина внутримышечно у животных в пределах 60 минут после дачи раствора развивался тяжелый отек легких. В случае дачи только раствора, вызывающего гиперволемию, или только питрессина, отека легких не наблюдалось. Развитие отека, по всей вероятности, вызывается совместным действием двух эффектов, а именно: уменьшения коллоидно-осмотического давления и повышения проницаемости легочных капилляров.

МНОГОКРАТНАЯ АДЕНОМА ОКОЛОЩИТОВИДНОЙ ЖЕЛЕЗЫ

Клинические, гистологические и электронномикроскопические исследования

Г. СИЛАДЬИ, И. БЕНЕДЕЦКИ и К. ЛАПИШ

Сообщается случай совместного наличия двойной аденомы околощитовидной железы и аденомы коры надпочечников.

Всвязи с подробными клиническими, гистологическими и электронномикроскопическими исследованиями наблюдаемого ими случая, а также удаленной оперативно аденомы, авторы доказывают, что «светлые основные клетки» обладают гормоносинтезирующей способностью.

Излагаются понятия о «третичном гиперпаратиреозе» и об «относительной гиперкальциурии». Всвязи с описанным случаем, авторы делают вывод, что раннее произведение операции непременно показано во всех случаях с подозрением на аденому, независимо от состояния почек.

АНАЛИТИЧЕСКОЕ ИССЛЕДОВАНИЕ ГАЗОВОГО ОБМЕНА КРОВИ У БОЛЬНЫХ С ЭРИТРЕМИЕЙ (POLYCYTHAEMIA VERA)

Д. НАДЬ, Я. СИЛАДЬИ. Ш. ОШВАТ и И. МЕРЦ

Авторы проводили у 37 больных эритремией в 82 случаях аналитическое измерение газового обмена крови. На основе полученных результатов в фазе обострения болезни удалось выявить отклонения, которые нормализовались в период ремиссии. По мнению авторов, указанные отклонения были вызваны увеличением числа эритроцитов и количества циркулирующей крови.

ИЗМЕНЕНИЯ ГЕМОДИНАМИКИ В СОСТОЯНИИ ШОКА ПОСЛЕ ЭКСПЕРИМЕНТАЛЬНОГО ИНФАРКТА МИОКАРДА

Д. ГАБОР, И. ЮХАС и Г. ПОГАЧА

Авторы создали экспериментальный склероз миокарда. В 15 из 22 экспериментов развился шок. Во всех случаях были исследованы изменения 1. минутного и систолического объемов крови, 2. кровяного давления, 3. периферического сопротивления, 4. коронарной и почечной фракций минутного объема крови, 5. коронарного и почечного кровотока и 6. сопротивления коронарных и почечных сосудов.

Результаты следующие:

1. Систолический и минутный объемы крови уменьшились в 19 случаях, в 12 из них развился шок.

2. В 7 случаях установленное рассчетом периферическое сопротивление повысилось до двухкратной величины по сравнению с исходной, причем в таких случаях шок не развился. Из 12 животных с шоком у 8 полное периферическое сопротивление показало лишь умеренное повышение. В остальных 4 случаях оно не изменилось или даже понизилось. У 3 животных шок развился, несмотря на неизменные величины систолического и минутного объемов крови. В этих случаях полное периферическое сопротивление показало значительный спад.

3. В 5 случаях были определены изменения минутного объема крови и сопротивления также через 30 и 60 мин. после развития некроза. Шок развился лишь в одном случае, причем полное периферическое сопротивление и систолический объем крови показали значительный спад. В остальных 4 экспериментах через 30 мин. после появления некроза минутный объем крови уменьшился, но по истечении 60 мин. он вновь достиг нормальной величины.

4. Линейные связи удалось выявить между а) коронарной фракцией минутного объема крови и полным периферическим сопротивлением, б) почечной фракцией минутного объема крови и полным периферическим сопротивлением и в) коронарной и почечной фракциями минутного объема крови.

5. Между константами коронарного и почечного кровотока и остальными параметрами кровообращения не было выявлено корреляции.

АНАЛИЗ ПАТОМЕХАНИЗМА НОЧНОГО НЕДЕРЖАНИЯ МОЧИ ЦЕРЕБРАЛЬНОГО ПРОИСХОЖДЕНИЯ ЭЛЕКТРОЭНЦЕФАЛОГРАФИЕЙ И ЦИСТОМЕТРИЕЙ

Ф. КАЙТОР, И. ОВАРИ и О. ЖАДАНЬИ

На спонтанной ЭЭГ без наркоза наблюдается замедление, иногда эпилептиформная активность. Наркоз почти в половине случаев активирует спайки в центральной и передней фронтальной областях; в некоторых случаях появляются спайк-волны и височный электрический очаг. Наркоз вызвал энурез в 77 процентах, а раздражение лишь чувствительной коры — в 9 процентах случаев произведения теста. В 14 процентах случаев не удалось осаждать мочу.

Энурез встречался в 83% случаев в фазе наркоза, характеризуемой дельта-активностью. Глубина наркоза, вызывающая мочеиспускание, повысила число спайков лишь в 17% случаев, в остальных случаях она подавляла спайки. Эпилептигенное повреждение мозга удалось доказать лишь изредка, но возможность повреждения мозга во время родов, или повреждения мозга другого происхождения, была установлена в 55 процентах случаев.

Авторы предполагают, что центральные или фронтальные спайки отражают ретардацию развития определенных неспецифических структур первичной чувствительно-двигательной коры, а также передней фронтальной коры, что играет патогенетическую роль в поддержании ночного недержания мочи.

МЕТАБОЛИЗМ ДЕГИДРОЭПИАНДРОСТЕРОНА В ЧЕЛОВЕЧЕСКОЙ КОЖЕ IN VITRO

И. ФАРЕДИН, Й. Л. ВЕББ и М. ЮЛЕС

Авторы вырезали у женщин куски кожи из области лобка и живота. Они инкубировали куски кожи в течение 5 часов в среде Кребс—Рингер—фосфат—глюкозы в присутствии дегидроэпиандростерона-4-C^{14}. При помощи хроматографии на столбе (окиси алюминия), на тонком слое (окиси алюминия, силика-геля G) и на бумаге удалось выявить многочисленные меченые продукты. Из инкубационной среды удалось извлечь меченые C^{14} андрост-4-ен-3,17-дион, 7-гидроксидегидроэпиандростерон, 7-кето-дегидроэпиандростерон и андростан-3,17-дион. Полученные результаты подтверждают то предположение, что кожа человека содержит такие энзиматические системы, которые способны вызвать метаболизм дегидроэпиандростерона in vitro.

СПКЕТРОФОТОМЕТРИЧЕСКИЕ ИССЛЕДОВАНИЯ АБСОРБЦИИ В УЛЬТРАФИОЛЕТОВОЙ ОБЛАСТИ

А. ЛАКОШ, А. ЯКИ и Л. ЛЕХОТАИ

В оформлении характерной спектрограммы (кривая интерференции) цереброспинальной жидкости, измеряемой в ультрафиолетовой области, аскорбиновая кислота играет решающую роль среди веществ, содержащихся в жидкости.

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INCREASED NEUROMUSCULAR EXCITABILITY IN NON-SPECIFIC RESPIRATORY DISEASES OF CHILDREN

By

MARIA CSERNOVSZKY, L. BRANYICZKY and VERA CSIKÓS

KÉKESTETŐ SANATORIUM (DIRECTOR: DR. L. BRANYICZKY)

(Received January 10, 1966)

Increased neuromuscular excitability has often been observed in 196 children, aged 3 to 14 years, suffering from allergic and other upper respiratory diseases. To elucidate the pathophysiology of the phenomenon the serum total calcium and inorganic phosphorus levels and the ECG have been studied.

In the conditions associated with bronchial spasm the most conspicuous change was a hyperphosphataemia (8.1 ± 0.3 mg per 100 ml in bronchial asthma, 7.74 ± 0.24 mg per 100 ml in asthmatic bronchitis), invariably present in the active phase, as well as ECG changes such as a prolonged Q—T, with peaked, narrow, tall T waves found in 50 per cent of the cases of bronchial asthma and in 38 per cent of those of asthmatic bronchitis.

The changes were presumably due to the decrease of the ionised diffusible calcium level developing together with the hyperphosphataemia. This change may enhance the action of nervous impulses on the small respiratory pathways, and thus one of the conditions of the development of an asthmatic attack may take an increased effect.

At our Sanatorium it belongs to the routine examination of children to elicit so-called tetanic reflexes such as CHWOSTEK's facial, PETÉNYI's femoral and LUST's peroneal reflex. Under 3 years of age their positivity is usually considered a diagnostic sign of latent tetany, mostly rachitic tetany. In older children tetany is less frequent and of different aetiology. The above-mentioned reflexes are positive in such cases, too, but are by themselves not pathognomonic. Opinions vary as to the cause and significance of their positivity; paediatricians usually consider a positive facial reflex to represent a sign of neuropathy [1, 2, 3].

In children suffering from allergic inflammatory respiratory disorders we could often elicit tetanic reflexes, and have made an attempt at the elucidation of the phenomenon.

Materials and methods

A total of 196 children, aged 3 to 14 years have been examined; 100 children suffered from bronchial asthma; 63 of asthmatic (allergic) bronchitis, and 33 of other respiratory tract disorders such as chronic bronchitis, bronchiectasis, pneumonia, and the sequelae to measles or whooping cough.

Apart from usual clinical examinations and laboratory tests we have determined the serum inorganic phosphorus according to FISKE and SUBBAROW [4], and total serum calcium by flame photometry. An ECG was made in every case [5, 6]. In every case we have attempted to elicit CHWOSTEK's facial, PETÉNYI's femoral and LUST's peroneal reflex.

Results

In *Fig. 1* is presented the patient material according to diseases. Of the patients 51 per cent suffered from bronchial asthma, 32 from asthmatic bronchitis and 17 per cent from other respiratory conditions. No age and sex differences could be demonstrated in the single groups.

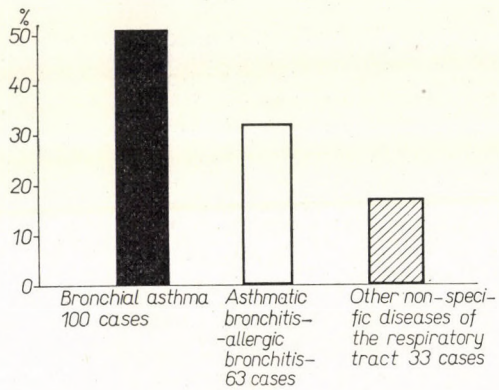

Fig. 1. Distribution according to disease

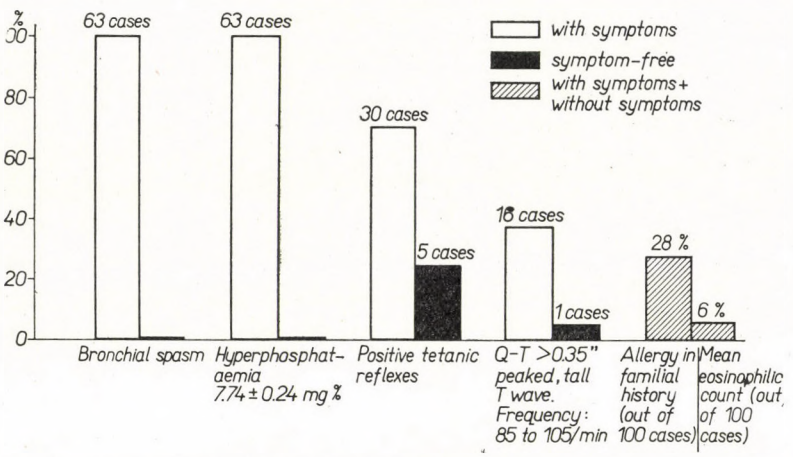

Fig. 2. Results of examinations in 100 children with bronchial asthma

Eosinophilia in children suffering from bronchial asthma averaged 7 per cent, in children with asthmatic bronchitis, 6 per cent, in the miscellaneous group, 2 per cent. In all three groups serum Ca was within the normal 9 to 11 mg per 100 ml range.

Fig. 2 contains the data for bronchial asthma patients. The patients displaying symptoms at the time of examination showed hyperphosphataemia.

The inorganic phosphorus value in these cases was 8.1 ± 0.3 mg per 100 ml, whereas in the cases free of symptoms, 3.7 ± 0.3 mg per 100 ml. The tetanic reflexes could be elicited in 75 per cent (in 42 cases out of 56) of the actually ill patients, as compared to the 20 per cent (9 cases out of 44) in the symptom-free group. The fourth column shows the incidence of ECG changes; Q—T exceeding 0.35 sec and peaked, narrow, tall T waves were found in 59 per cent (in 33 cases out of 56) of the patients with symptoms, as compared to the mere 7 per cent (3 cases ouf of 44) in the symptom-free group. Heart rate averaged 85 to 105/min., thus the electric systole as related to frequency, was prolonged.

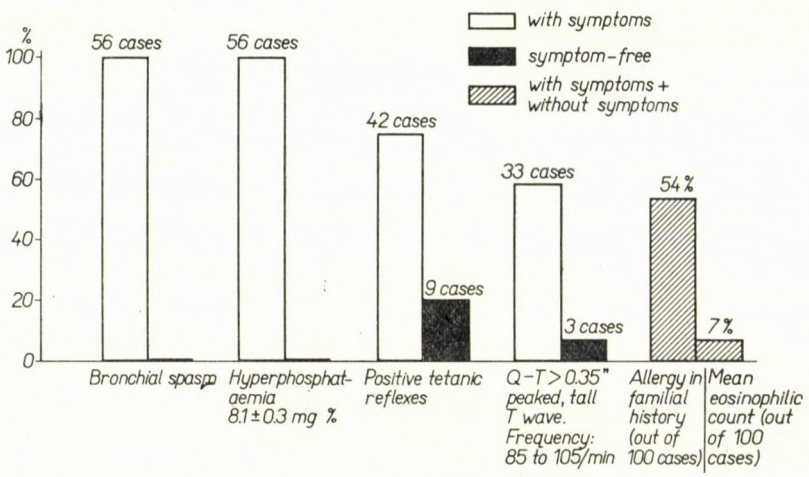

Fig. 3. Results of examinations in 63 children with asthmatic bronchitis

In *Fig. 3.* are presented the data for children suffering from asthmatic bronchitis. In the cases showing symptoms at the time of examination the serum inorganic phosphorus level was 7.74 ± 0.24 mg per 100 ml, as compared to the 4.1 ± 0.27 mg per 100 ml in the symptom-free group. When there were symptoms of bronchitis, tetanic reflexes could be elicited in 71 per cent (in 30 cases out of 42), whereas in the symptom-free group they could be evoked in 24 per cent (in 5 cases out of 21). ECG changes (longer than 35 sec and peaked, narrow, tall T) could be demonstrated in 38 per cent (16 cases of the 42) of those showing symptoms, as compared to just 5 per cent (1 of the 21) symptomless at the time of study. In this group, too, the heart rate averaged 85 to 105/min.

Fig. 4 shows the relationship of tetanic symptoms to the serum inorganic phosphorus level. In the cases showing symptoms at the time of examination the signs of increased neuromuscular excitability, bronchial spasm, positive tetanic reflexes and ECG changes occurred together with hyperphosphataemia whereas in the symptom-free group, if they occurred at all, they were not associated with hyperphosphataemia.

In the miscellaneous group, serum inorganic phosphorus was 4.8 ± 0.43 mg per 100 ml. No ECG changes could be demonstrated. Tetanic reflexes could be elicited in 9 per cent (3 cases out of 33).

The results of all three groups are summarized in *Table I.*

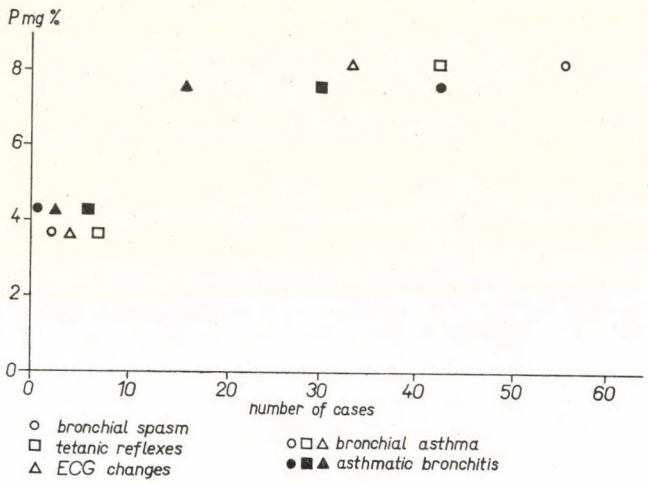

Fig. 4. Correlation between tetanic symptoms and serum inorganic P level

Table 1

Disease group	Number of cases	Serum total Ca mg per 100 ml	Serum inorg. P mg per 100 ml	Positive tetanic reflexes	ECG changes	Broncho-spasm	Allergy in familial history	Mean eosinophil count
Bronchial asthma with symptoms	56	9 to 11	8.1 ±0.3	75% 42 cases	59% 33 cases	100% 56 cases	54%	7%
without symptoms	44	9 to 11	3.7 ±0.31	20% 9 cases	7% 3 cases	7% 3 cases	with and without symptoms together	
Asthmatic bronchitis with symptoms	42	9 to 11	7.74 ±0.24	71% 30 cases	38% 16 cases	100% 42 cases	28%	6%
without symptoms	21	9 to 11	4.1 ±0.27	24% 5 cases	5% 1 case	5% 1 case	with and without symptoms together	
Nonspecific respiratory diseases with symptoms	33	9 to 11	4.8 ±0.43	9% 3 cases	0%	6% 2 cases	2%	2%

Note. In the asthmatic bronchitis and the miscellaneous groups, symptoms of inflammation and fever were also noted.

In children with bronchial asthma, other allergic manifestations (eczema, urticaria) alternated, in the midst of complete well-being, with sudden episodes of expiratory dyspnoea. In the family of these patients there was evidence of allergic diseases in 54 per cent.

In patients with allergic bronchitis inflammatory symptoms such as leukocytosis, subfebrility, fever, increased erythrocyte sedimentation rate, cough, expectoration could be observed invariably when asthmatic symptoms were present. In the family of these patients, allergic diseases occurred in 28 per cent.

Discussion

As to the effect of calcium on the respiratory tract, WARNANT [7] described the bronchoconstrictive action of calciums salts, while POTTENGER [8] claimed them to be bronchodilator agents. POTTENGER recommended calcium salts for the treatment of asthmatic attacks and reported on favourable results. Since then, clinical observations have corroborated his claim. The intravenous administration of calcium salts elevates the level of biologically active, ionised calcium.

Serum calcium, phosphorus, magnesium and potassium determinations in allergic conditions (bronchial asthma, angioneurotic oedema, eczema) were reported by HAJÓS [9], and more recently by BERNHEIM [10]. Not only the absolute, but also the relative serum calcium, magnesium, potassium, sodium and phosphorus values are of decisive importance. According to the formula of GYÖRGY [11]

$$K = \frac{[Na^+] + [K^+]}{[Ca^{++}] + [Mg^{++}] + [H^+]}$$

the increase of the K (constant) is accompanied by an increase, its decrease by a decrease, of excitability. The increase of the concentration of monovalent ions, or the decrease of that of bivalent ones enhances the excitability, the tendency to convulsions. Our cases could be characterized by György's formula, as we did not determine the Mg^{++} and H^+ levels in any of the cases. Neither were the Na^+ and K^+ levels determined in every case.

As has been pointed out by GERLÓCZY [12], the elevation of the serum potassium level is of a tetanogenic action, by altering the normal Ca/K ratio.

A shift in the direction of P in the normal Ca : P = 2 : 1 ratio may lead to latent tetany even with a normal serum calcium level. By latent tetany, QUANDT and PONSOLD [13] mean a condition in which a true hypocalcaemic state develops, while the serum total calcium level is normal. Upper respiratory catarrhs may often lead to latent tetany.

As to the pathomechanism of the changes observed by us the following hypothesis may be put forward. During the period when the conditions associated with bronchospasm produce symptoms, the most conspicuous findings in the asthmatic and allergic bronchitis groups were hyperphosphataemia in a significant part of the cases (59 and 38 per cent, respectively), ECG changes indicative of a decrease in the ionised calcium level of serum, further, positive tetanic reflexes in 75 and 71 per cent, respectively. In the symptomless period the bronchospasm ceases, the children show serum inorganic P levels lower than normal, ECG changes occur in 7 and 5 per cent, respectively, positive tetanic reflexes in 20 and 24 per cent, respectively.

The changes found in the period displaying symptoms could be interpreted by a decrease of parathyroid activity, had the serum calcium values been low. Since they were normal, we may only suggest the possibility that the ionised calcium fraction was decreased.

According to GÜNZEL [14], LUTTEROTTI and KORTH [15], as well as DOXIADES and VOLLMER [16], in the diagnosis of latent tetany the behaviour of the Q—T segment is a decisive point which may replace the determination of the serum ionised calcium level. According to KAISER [17], however, it is only with serum total calcium levels below 7.4 mg per 100 ml that a prolongation of Q—T results with great probability, but in most cases of relative parathyroid insufficiency hypocalcaemia is not so severe. According to MOSONYI [18] hypoparathyroidism must not be accompanied by hypocalcaemia. The biological activity of the ionised calcium fraction is the decisive factor; unfortunately, clinical methods for the routine determination of the ionised calcium level are still lacking.

Various causes might explain the elevated serum inorganic P level observed in every case during the symptomatic period, such as

(i) disturbances of parathyroid function,
(ii) renal diseases,
(iii) increased breakdown of high-energy phosphate esters.

(i) In children displaying symptoms the bronchospasm may be interpreted as a sign of visceral tetany, and the ECG changes, as well as the positive tetanic reflexes as symptoms of latent tetany. On the basis of the results we cannot, however, decide with certainty which was the primary change, the relative parathyroid insufficiency, or the transitory increase of the serum inorganic P level not due to parathyroid activity.

(ii) Renal function was normal in every one of the 196 children, so that renal diseases could be ruled out as a cause of hyperphosphataemia.

(iii) The serum inorganic P level may increase in consequence of an increased breakdown of ATP due to dyspnoea — hypoxia —, or to some other mechanism. MÁNYAI [19] found a decrease of ATP concentration in erythro-

cytes in bronchial asthma. GEORGE *et al.* [20] demonstrated a decrease of the serum inorganic P level following 30 minutes hyperventilation. At the same time, the acid-soluble organic P level significantly increased in the erythrocytes. Part of the serum inorganic P loss appeared in the high-energy phosphate esters of erythrocytes. These data have a clinical importance. Investigations of this kind may elucidate the pathomechanism of the bronchospasm producing the same symptoms in various pathological conditions, and may also help in finding the causal therapy.

As to the serum inorganic P values in the symptom-free children suffering from allergic respiratory diseases, these values were lower than those found in other affections of the respiratory organs.

MOSONYI and ÁCS [21, 22] have pointed out the important fact that following prolonged administration of drugs of cortisol action hypocalcaemia may develop when treatment is discontinued. During treatment calcium absorption is inhibited. This leads to an increase of parathyroid activity, which persists after the treatment has been discontinued. This protracted hyperactivity may explain the low serum inorganic P levels found in the patients free from symptoms.

On the basis of the observed changes and the data in the literature a relative parathyroid failure may be assumed to occur in the symptomatic period of bronchial asthma and asthmatic bronchitis. The ensuring hyperphosphataemia enhances parathyroid function through a feed-back mechanism. Parathyroid activity may be so enhanced that it may keep the serum total calcium at a normal level. However, the hyperphosphataemia then reduces the serum ionised calcium level, and thus latent tetany may develop. The increase in compensatory parathyroid activity, further promoted by steroid treatment, contributes to the abolition of the observed changes.

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ZUR HÄMODYNAMIK DER ESSENTIELLEN HYPERTONIE*

Von

R. JUCHEMS

MEDIZINISCHE POLIKLINIK (DIREKTOR: PROF. DR. H. FRANKE) DER UNIVERSITÄT WÜRZBURG

(Eingegangen am 13. Juli, 1966)

Es wird über experimentelle Untersuchungen mittels der Farbstoffverdünnungsmethode an 20 Hypertonikern und einer Gruppe Gesunder, sowie Patienten mit neurozirkulatorischer Asthenie berichtet. Der periphere Widerstand war in der Hypertonikergruppe erhöht, in der Patientengruppe mit funktionellen Herzleiden vermindert. Herzminutenvolumen und Schlagvolumen waren bei den Patienten mit essentieller Hypertonie vermindert, unter Berücksichtigung des Altersunterschiedes, mit Ausnahme der dekompensierten Fälle aber normal.

Seit der Einführung von Indikatorverdünnungsverfahren in die klinische Forschung haben wir eine Methode zur Verfügung, die es erlaubt, ohne Gefährdung und wesentliche Belastung des Patienten exakte Messungen des Herzminutenvolumens, Schlagvolumens und peripheren Gesamtwiderstandes am Menschen durchzuführen [9, 11, 14]. Das Prinzip des Verfahrens darf ich kurz damit charakterisieren, daß wir nach Injektion eines Indikators in die venöse Strombahn des Menschen an der arteriellen Seite mit modernen Apparaturen blutig die Konzentration fortlaufend messen und aus dem Zeit-Konzentrations-Integral das Herzminutenvolumen errechnen.

Über die Hämodynamik der essentiellen Hypertonie liegen zum Teil sich widersprechende Befunde vor. Mehrere Untersucher [3, 8, 20] konnten eine Erhöhung des Herzminutenvolumens nachweisen; andere fanden eine Abnahme [10, 21]. Schließlich wurde von manchen Forschern [1, 4, 16] keine Änderung des Herzminutenvolumens gesehen. Auch wurde je nach dem Schweregrad der Hypertonie ein vermindertes oder erhöhtes Herzminutenvolumen beobachtet. ROWE [17] fand bei Schweregrad 1 und 2 einen normalen Herzindex, bei Schweregrad 3 und 4 jedoch einen verminderten. Ein erhöhter peripherer Gesamtwiderstand wurde mit unterschiedlichen Methoden von verschiedenen Arbeitsgruppen mitgeteilt [1, 3, 8, 10, 16, 17, 19, 21].

Methodik

Unsere Untersuchungen wurden mittels eines neuartigen Densitometers der Firma Waters (Rochester, Minnesota, USA) bei Verwendung einer Durchflußküvette durchgeführt. Die Apparatur weist die Farbstoffkonzentration des Indikators Cardiogreen bis zu einer Konzentration von 48 mg pro l linear an. Als Schreibgerät verwandten wir den Schreiber BD—3

* Vortrag gehalten an der Jahrestagung der Ungarischen Gesellschaft für Kardiologie Balatonfüred, 14. Mai 1966.

der Firma Kipp & Zonen, Delft, Holland. Nach Punktion der arteria femoralis und Anschluß an die Küvette wurde eine Verweilküvette in die rechte vena cubitalis eingelegt.

Um das Blut ungerinnbar zu machen, spritzten wir 25 000 Einheiten Heparin ein. Danach injizierten wir mit Spezialspritzen 5 mg Cardiogreen in die Vena cubitalis des Probanden. Die Originalfarbstoffverdünnungskurve wurde auf semilogarithmisches Papier übertragen und

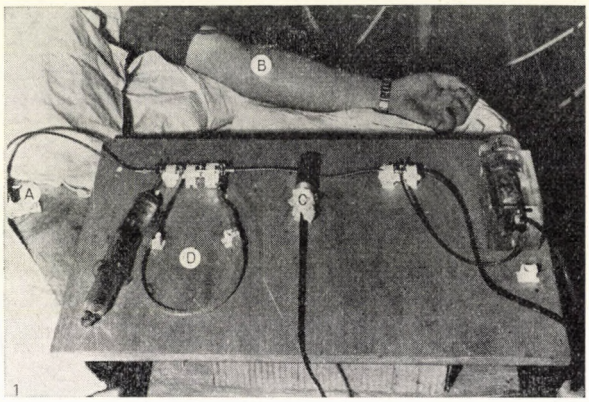

Abb. 1. Anordnung zur Messung des Herzminutenvolumens nach dem Indikatorverdünnungsverfahren. Punktion der Art. femoralis (A) und der Vena cubitalis (B) und Anlegen von Verweilkanülen. Ein PVC-Schlauch mit einem Durchmesser von 4 mm verbindet die Arterienpunktionsnadel mit der Durchflußküvette (C). Zwischen Arterie und Küvette kann ein Modellkreislauf, der mit silikonisierten Glaskugeln angefüllt ist, eingeschaltet werden (D), womit eine dynamische Eichung der Indikatorverdünnungskurve erzielt wird

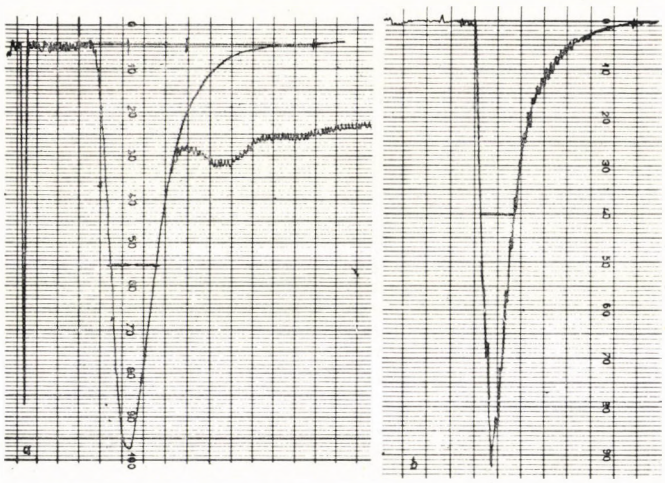

Abb. 2. a) Originalfarbstoffverdünnungskurve nach Injektion von 5 mg Cardiogreen. Die Größe des Herzminutenvolumens ist umgekehrt proportional der Fläche der Primärkurve. *b)* Eichkurve mittels Modellkreislauf

nach dem Vorgehen von KINSMAN, MOORE und HAMILTON die Rezirkulation ausgeschlossen [15]. Aus der gewonnenen Primärkurve wird mittels dynamischer Eichung [18] die Farbstoffkonzentration und das Herzminutenvolumen errechnet.

Das Schlagvolumen erhielten wir durch Division des Herzminutenvolumens durch die Anzahl der Zacken in der Farbstoffverdünnungskurve pro min. (Siehe Abb. 2a).

Doppelmessungen des Herzminutenvolumens und Schlagvolumens hatten eine gute Übereinstimmung der Meßwerte gezeigt ($r = 0{,}93$). Einzelheiten sind an anderer Stelle beschrieben [11].

Der periphere Widerstand errechnet sich durch Multiplikation des mittleren Blutdruckes mit 1332, dividiert durch das Stromvolumen in der Sekunde. Als mittlerer Blutdruck gilt diastolischer Blutdruck, plus 1/3 der Blutdruckamplitude. Die Untersuchungen wurden alle am liegenden Patienten in einem thermoindifferenten Milieu, bei einer Durchschnittstemperatur von 20° C durchgeführt. Zum Vergleich wurden Herzgesunde und Patienten mit neurozirkulatorischer Asthenie herangezogen; letztere zeigen nicht selten einen labilen Hochdruck. Den folgenden Ergebnissen liegen Untersuchungen an 15 Normalpersonen, 15 Patienten mit neurozirkulatorischer Asthenie und 20 Hypertonikern zugrunde. Bei letzteren war ausnahmslos der *Hochdruck über 5 Jahre* bekannt. Statistische Untersuchungen auf Signifikanz wurden nach dem Student-Verfahren durchgeführt. Als signifikant gilt, wenn die Überschreitungswahrscheinlichkeit unter 0,05 liegt.

Ergebnisse

Der Durchschnittswert für das Herzminutenvolumen war in der Hypertonikergruppe 4,39 l/min ($s = \pm 0{,}68$); die Normalpersonen zeigten ein Herzminutenvolumen von 5,31 l/min ($s = \pm 0{,}71$). Die Patienten mit neurozirkulatorischer Asthenie hatten schließlich ein Herzminutenvolumen mit durchschnittlich 6,75 l/min ($s = \pm 1{,}2$). Auch der periphere Widerstand war in den verschiedenen Gruppen unterschiedlich. Den höchsten Mittelwert zeigte die Hypertonikergruppe mit durchschnittlich 2298 dyn · sec · cm^{-5}. Der Normalwert betrug 1481 dyn · sec · cm^{-5} ($s = \pm 294$). Die Patienten mit neurozirkulatorischer Asthenie hatten im Durchschnitt einen geringeren peripheren Widerstand mit 1161 dyn · sec · cm^{-5} ($s = \pm 248$). Das Schlagvolumen zeigte auch ein unterschiedliches Verhalten. Der Durchschnittswert für Normalpersonen betrug 72 ml, für Hypertoniker 58 ml und für die Patienten-

Tabelle 1

Mittelwerte und Standardabweichung des Mittelwertes verschiedener hämodynamischer Parameter bei Hypertonikern und Normalpersonen

	Normalpersonen (n = 15)	Hypertoniker (n = 20)
Alter	33,2 ($s_m = 3{,}08$)	46,01 ($s_m = 2{,}56$)
Länge	168,2 ($s_m = 1{,}68$)	169,1 ($s_m = 1{,}42$)
Gewicht	68,0 ($s_m = 2{,}71$)	76,0 ($s_m = 2{,}34$)
Herzminutenvolumen	5,31 ($s_m = 0{,}18$)	4,39 ($s_m = 0{,}15$)
Herzindex	2,97 ($s_m = 0{,}09$)	2,31 ($s_m = 0{,}08$)
Schlagvolumen	72,1 ($s_m = 3{,}48$)	58,1 ($s_m = 2{,}56$)
Herzfrequenz	73,5 ($s_m = 1{,}82$)	76,8 ($s_m = 2{,}28$)
Mittlerer Blutdruck	96,6 ($s_m = 2{,}96$)	127,5 ($s_m = 2{,}51$)
Peripherer Widerstand	1481,2 ($s_m = 75{,}5$)	2297,7 ($s_m = 95{,}8$)

s_m = Standardabweichung des Mittelwertes

gruppe mit neurozirkulatorischer Asthenie durchschnittlich 84 ml. Die Einzelwerte in der letzten Gruppe zeigten Erhöhungen bis 118 ml. Der mittlere Blutdruck betrug durchschnittlich 127 mmHg in der Patientengruppe mit Hypertonie, 96,6 mmHg bei den normalen Versuchspersonen, und war bei den Patienten mit neurozirkulatorischer Asthenie nicht signifikant abweichend.

Diskussion

Unsere Ergebnisse der Schlagvolumen- und Herzminutenbestimmung an den Normalpersonen lassen sich mit den Werten anderer Autoren gut vergleichen. HEGGLIN beobachtete ein durchschnittliches Herzminutenvolumen bei Herzgesunden von 5,5 $\pm$ 0,75 l/min [9], HOCHREIN und SCHNEIDER 6,2 l/min [10]. NICHOLSON und WOOD [14] geben einen Herzindex von 3,17 an, DOYLE et al. [5] erhielten 3,2 an 53 Herzgesunden.

Statistische Berechnungen zeigten einen signifikanten Unterschied des Herzminutenvolumens der Hypertonikergruppe vom Normalkollektiv ($P < 0{,}001$). Der Herzindex ist im Vergleich beider untersuchten Gruppen (Normalpersonen und Hypertoniker) noch deutlicher unterschieden, da die Hypertoniker eine Übergewichtigkeit zeigten. Eine Adipositas ist bei Hypertonikern häufig anzutreffen, wie schon frühere Autoren [21] berichtet haben. Da bei unseren *Hypertonikern das Leiden mindestens fünf Jahre bekannt war* und mehrere Patienten einen Fundus hypertonicus (Stadien III—IV nach KEITH und WAGENER) zeigten, können wir unsere Ergebnisse gut mit den Befunden von ROWE vergleichen, der bei fortgeschrittener Hypertonie ebenfalls einen reduzierten Herzindex fand [17]. Auch WOLLHEIM hatte dieses Verhalten

Tabelle 2

Ergebnisse der Signifikanzberechnungen nach dem Studentverfahren. Der Unterschied gilt statistisch signifikant, wenn die Überschreitungswahrscheinlichkeit (P) 0,05 oder kleiner ist

	$\bar{x}_1$	$\bar{x}_2$	t	2 P
Alter	33,2	46,01	3,2	0,01
Körperlänge	168,2	169,1	0,372	0,7
Gewicht	68,0	76,0	2,247	0,02
Herzfrequenz	73,47	76,8	1,038	0,3
HMV	5,31	4,39	3,833	0,001
Schlagvolumen	72,07	58,05	3,302	0,01
Herzindex	2,97	2,31	6,642	0,001
P_m	96,55	127,5	8,068	0,001
Peripherer Widerstand	1481	2298	6,371	0,001

nachweisen können [21]. Es fragt sich jedoch, ob nicht der Altersfaktor ein vermindertes Herzminutenvolumen vortäuscht. Unsere Patienten waren im Durchschnitt 46 Jahre alt und damit 13 Jahre älter als die Normalpersonen. Schon WEZLER hat auf eine Altersabhängigkeit des Zeitvolumens hingewiesen [19].

Neuere Untersuchungen von BRANDFONBRENER et al. [2] mit dem Indikatorverdünnungsverfahren haben für 24jährige ein durchschnittliches Herzminutenvolumen von 6,49 l/min, für 55jährige aber von 4,63 l/min nachweisen können. Eigene Untersuchungsergebnisse an weiteren Hypertonikern zeigten im Durchschnitt bei 24jährigen ein Zeitvolumen von 6,5 l/min, eine Patientengruppe mit einem Alter um 53 Jahre hatte hingegen ein Herzminutenvolumen von 4,95 l/min [13]. Es kann somit angenommen werden, daß das Herzminutenvolumen bei Hypertonikern altersabhängig ist; möglicherweise dürfte aber auch die Dauer der Erkrankung von Bedeutung sein.

Unsere Patienten mit funktionellen Herz- und Kreislaufstörungen hatten ein gesteigertes Herzminutenvolumen bei erhöhtem Herzindex. Ihr mittlerer Blutdruck war nicht von dem der Normalpersonen unterschieden. An die Messung des Blutdruckes wurden strenge Kriterien angelegt und der Einfluß psychischer Faktoren auf den Blutdruck wurde möglichst ausgeschlossen. Es ist ja bekannt, daß unter der Diagnose »funktionelle Herzkrankheit« sich Patienten finden, die zeitweise Blutdruckerhöhungen in Form eines »labilen« Hochdruckes aufweisen. Es ist zu diskutieren, ob nicht die aus der Literatur her bekannten erhöhten Herzminutenvolumina bei Hypertonikern durch Einbeziehung von Patienten mit labilem Hochdruck vorgetäuscht werden. FINKIELMAN [6] hat kürzlich einen Anstieg des Zeitvolumens bei Patienten mit labilem Hochdruck nachgewiesen. Die Patienten mit *Hochdruck und Herzdekompensation* jedoch zeigten ein stärker vermindertes Herzminutenvolumen im Vergleich mit den kompensierten Hypertonikern, wie das auch zu erwarten war.

Die Herzfrequenz war in der Hochdruckgruppe nicht wesentlich verschieden, jedoch bei den Patienten mit neurozirkulatorischer Asthenie leicht erhöht. Das Schlagvolumen, das bei unseren Probanden eine deutliche Korrelation mit dem Herzminutenvolumen zeigte, war in der Hypertonikergruppe im Durchschnitt vermindert.

Ein Anstieg des peripheren Widerstandes bei Hypertonikern wurde von mehreren Forschern beobachtet [1, 3, 17, 19, 20, 21 u. a.]. Unsere Patienten mit Hypertonie zeigten eine signifikante Erhöhung des peripheren Widerstandes ($P < 0{,}001$). Dabei war der mittlere Druck mit dem erhöhten peripheren Widerstand korreliert. Der periphere Widerstand war bei den Untersuchten mit neurozirkulatorischer Asthenie vermindert. Im Experiment konnten BROD et al. durch Stimulation das Hypothalamus, bzw. der cerebralen Cortex mit nachfolgender Aktivation der Muskulatur eine Erhöhung des peripheren Widerstandes verursachen [3]. Diese Ergebnisse haben diese Autoren

zu der These angeregt, die veränderte Hämodynamik bei Hypertonikern entspreche den Kreislaufumstellungen von Normalpersonen, die eine anstrengende körperliche Tätigkeit ausüben wollen oder sich für körperliches Training vorbereiten. FOLKOW [7] fand eine verstärkte Konstriktion der Arteriolen im Anfangsstadium der Hypertonie. Als Folge davon sollte die Erhöhung des Druckes nach anfänglicher Steigerung zu einer Verminderung des Herzminutenvolumens und der Herzarbeit führen, da diese annähernd proportional dem Quadrat des Herzminutenvolumens ist.

Letzten Endes sind die Theorien über die Entstehung des veränderten peripheren Widerstandes bei Hypertonie gegenwärtig noch zu sehr spekulativ, als daß wir endgültig dazu Stellung nehmen könnten.

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HAEMODYNAMICAL SIGNIFICANCE OF VENOUS BRONCHOPULMONARY CIRCULATION

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The role played by the venous bronchopulmonary anastomoses of the lung under normal haemodynamical conditions is not clear. When there is hypertension in the system of the pulmonary vein (e.g. in mitral valvular disease) the major part of pulmonary arterial blood is diverted through the bronchial veins into the azygos vein or right atrium, without having flown through the left heart. It is likely that this shunt reduces to some extent the congestion in the pulmonary venous system, but at the same time increases the burden placed upon the right heart. The increased rate of bronchopulmonary venous flow may be demonstrated by catheterisation of the azygos vein.

In spite of the advances achieved in the field of haemodynamical examinations, many aspects of the bronchopulmonary shunts remain difficult to approach. The role of the arterial connexions between the pulmonary and systemic circulations (the bronchopulmonary arterial shunts) is explained by the evidence obtained in valvular heart diseases associated with reduced pulmonary blood flow, but VIRCHOW's observations [1] could only be supplemented by some further cases, without allowing an insight into the background of the haemodynamical changes. For example, in Fallot's tetralogy the low pulmonary cardiac output is followed in certain cases by a dilatation of the systemic bronchial arteries, and through the bronchopulmonary shunts the rate of pulmonary blood flow increases. It remains, however, unknown why this more or less compensatory mechanism develops in some, but not in all cases.

Our knowledge concerning the venous shunts of the lung is still more defective. The venous system of the lung is composed of the following types of vein.

1. Pulmonary vein, draining the alveolar capillary network and emptying into the left atrium; it carries arterial blood.

2. True bronchial veins are the veins of the primary, secondary and tertiary bronchi. They are not directly connected with the pulmonary venous system, and through the intercostal veins or azygos vein they carry venous blood into the right atrium.

3. Bronchopulmonary veins [2] originate partly under the mucosa and partly under the muscular layer of the small bronchi, by-pass the alveolar network, and carry venous blood through the left pulmonary vein into the left atrium.

4. Anastomoses (shunts) between the bronchial and the pulmonary veins are capillary-sized, and are to be found in the region of the respiratory bronchiole. They empty in the direction of the true bronchial veins, thus, they carry arterial blood into the right heart.

In studies concerning the venous side of bronchopulmonary circulation [4, 6, 7] partial ligation of the pulmonary veins is usually carried out. The results are unequivocal: the bronchopulmonary shunts open up, and from the pulmonary artery part of the blood volume flows back into the right heart without having passed through the systemic circulation.

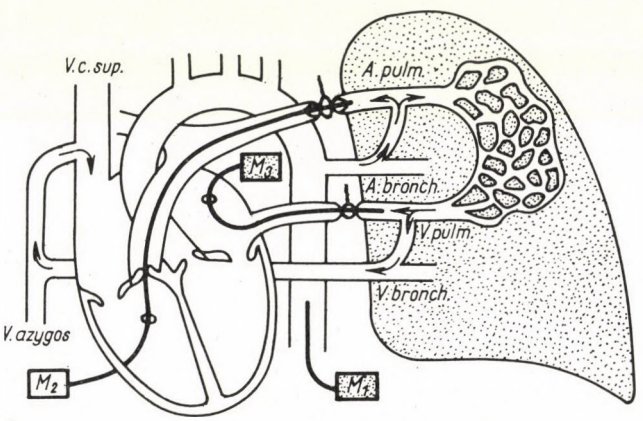

Fig. 1. Diagrammatic representation of the animal experiments. The artery and veins of the left lung are clamped. Manometer M_1 measures systemic pressure in the femoral artery, M_2 measures pressure in the pulmonary artery distal from the site of clamping, M_3 measures pressure in the pulmonary vein distal from the site of clamping

In the present study, the above observations have been supplemented by oxymetric and manometric data obtained in animal experiments and in patients displaying pathological changes in the pulmonary circulation.

Materials, methods and results

In 8 dogs under intratracheal anaesthesia and breathing a mixture of O_2 and halothane the chest and the pericardium were incised, opened and rubber cuffs were placed on the left pulmonary artery and vein. Through the apex of the right ventricle a Cournand catheter with a balloon was inserted into the left branch of the pulmonary artery. Through the left auricle polyethylene catheters were passed up into the left pulmonary veins. To stop the functional flow in the left lung, the blood vessels mentioned were pressed against the catheters in their lumen by inflating the cuffs placed under them and the pressure relations of the nutritive (bronchial) circulation were examined by the aid of the catheters.

Systemic pressure was measured by a manometer inserted into the right femoral artery (*Fig. 1*, M_1). Blood pressures distal from the clamps placed on the left pulmonary artery (*Fig. 1*, M_2) and pulmonary veins (*Fig. 1*, M_3) were measured by electromanometers. Before obstructing the blood vessel systems the pressures in them were determined (*Fig. 2*). Following clamping the artery and veins of the left lung, distal to the site of clamping pressure decreased to a few mm Hg and the arterial and venous pressure values became similar. The pressure curve of the pulmonary artery, too, became similar to a venous pressure curve, and its values, together with those of the pulmonary vein, corresponded to the pressure value measured in the right atrium (*Fig. 3*).

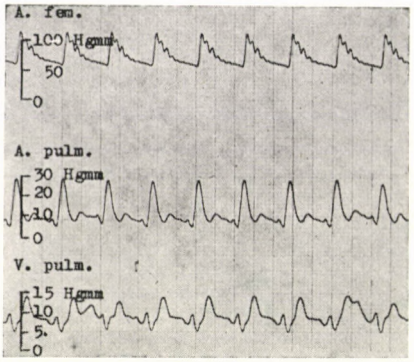

Fig. 2. Normal pressure values in the dog during general anaesthesia

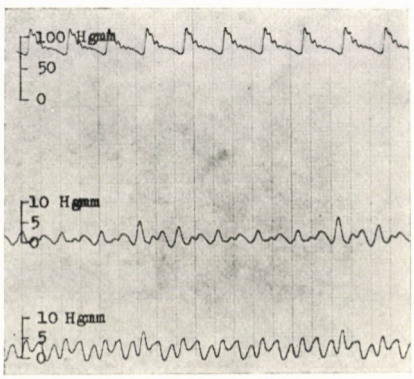

Fig. 3. Changes in the pressure values shown in *Fig. 2.* Above: femoral arterial pressure. Centre: pulmonary arterial pressure distal from the site of clamping. Below: pulmonary venous pressure distal from the site of clamping

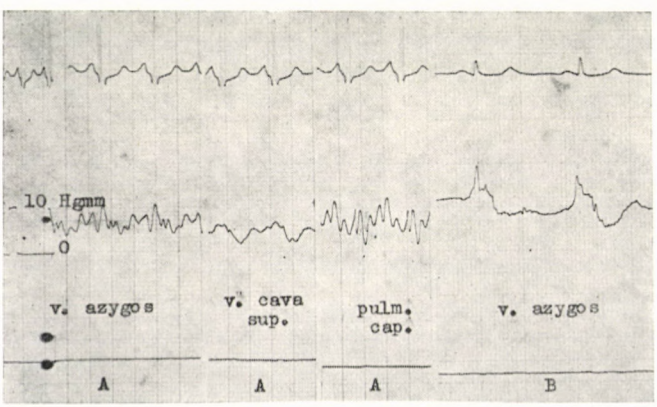

Fig. 4. Normal pressure values measured by catheterisation in the azygos vein, superior vena cava and pulmonary capillary in Case 10, Table I(A). Elevated pressure in the azygos vein in mitral stenosis, in Case 2, Table I(B)

Studies were carried out in six patients suffering from mitral disease accompanied by pulmonary venous hypertension, and in six others with valvular disease not causing pulmonary venous hypertension. When the right heart was catheterized, pressure was measured in the venae cavae, right atrium and ventricle, pulmonary artery and pulmonary capillaries, and in the azygos vein; oxygen saturation of blood was also determined (*Table I*).

In the thirteenth case, the pulmonary artery and post mortem the bronchial veins of the left lung were filled up with a 5 per cent polyvinyl solution [3], then the preparation was corroded. The arteries of the lower lobe were filled up but, as a result of the pulmonary embolisation which had taken place, the arterial system of the middle lobe could not be injected. Through the two big bronchial veins running in the hilum the pulmonary veins of the middle lobe were also filled up.

Discussion

Under normal haemodynamical conditions, the connexions between the pulmonary and bronchial blood vessels apparently represent no substantial shunt volume. However, the experiments conducted by MILLER in 1906 [4] have shown that under pathological conditions the functionally closed shunts would open up. Miller injected a gelatin solution stained with Prussian blue into the pulmonary artery; the gelatin passed over into the pulmonary veins but after the veins had been clamped, it appeared in the bronchial veins. Berry and Daly [5] perfused the pulmonary artery in dogs and found that about 1 per cent of the pulmonary arterial outflow per minute had flown toward the right atrium. According to AVIADO et al. [6] 7.3 to 8 per cent of the pulmonary arterial flow passes into the right atrium. ARAMENDIA et al. [7] perfused in heart-lung preparations the pulmonary artery and maintained the bronchial arterial circulation, and determined under such conditions the venous inflow into the right atrium. When the bronchial arteries had been clamped, bronchial venous back-flow diminished by 30 to 50 per cent. It was concluded that about 50 to 70 per cent of the bronchial venous volume originated from the pulmonary circulation.

In cases of pulmonary venous hypertension (such as occurs for instance in mitral stenosis) MARCHAND et al. [8] found that a substantial part of the pulmonary venous blood volume was diverted through the bronchial veins into the right atrium.

Our animal experiments have confirmed the existence of a bronchopulmonary connexion between the right and left atrium. After the pulmonary circulation of the left lung had been eliminated, this system could get blood exclusively from the bronchial arteries. The fact that distal from the obstruction right atrial pressure values were recorded not only in the pulmonary vein, but also in the pulmonary artery indicates that the pulmonary arterial system is connected through bronchopulmonary shunts with the right atrial one (pulmonary artery — pulmonary capillary — pulmonary vein — venous bronchopulmonary anastomosis — bronchial vein — azygos vein — superior vena cava — right atrium).

Table I

Case No.	Name	Diagnosis	Pressure, mm Hg, in				Oxygen saturation, per cent		Findings at operation
			Azygos vein	Right atrium	Pulm. artery	Pulm. art. wedge or left atrium	Azygos vein	Sup. vena cava	
1	Mrs. P. J.	Mitral stenosis	15/12	8/4	35/20	28/18	72.5	64.5	Mitral orifice of fingertip size
2	Mrs. B. Gy.	Mitral stenosis	18/12	15/10	—*	—	65	55	Mitral orifice, 0,6 sq. cm
3	K. I.	Combined mitral disease	20/8	15/6	60/40	50/30	69	60	—
4	B. N. M.	Combined mitral disease	30/12	10/3	58/25	35/14	—	—	Mitral orifice, 0,8 sq. cm
5	Sz. J.	Combined mitral disease	22/18	4/2	45/15	—	91	80.5	—
6	K. J.	Combined mitral disease	22/14	14/8	110/50	60/40	—	—	—
7	B. I.	Atrial septal defect	7/3	6/3	50/20	24/10	82.5	78.5	Atrial septal defect, measuring 2 sq. cm
8	Sz. S.	Atrial septal defect	7/4	6/2	18/5	15/5	—	—	Atrial septal defect measuring 3 sq. cm
9	P. A.	Atrial septal defect and pulmonary venous anomaly	8/5	7/1	25/12	10/7	76	72	Atrial septal defect, measuring 1 sq. cm; veins of the superior lobe of right lung opening into superior vena cava
10	P. V.	Ventricular septal defect	8/5	7/3	20/12	10/4	—	—	—
11	T. J.	Pulmonary stenosis	5/3	3/1	25/12	14/8	80	76.5	—
12	P. L.	Patent foramen ovale	8/4	5/1	25/12	15/6	82	79	—
13	Mrs. K. F.	Combined mitral disease, pulmonary embolism	—	14/7	—**	—	—	—	—

* Catheter could not be inserted into pulmonary artery. Pressure in right ventricle was 70/6 mm Hg.
** Catheter could not be inserted into pulmonary artery. Pressure in right ventricle was 50/4 mm Hg.

The above relations are corroborated by the evidence obtained on catheterisation of the azygos vein. In the control group (*Table I*, patients 7 to 12) pressure in the azygos vein was only a few mm Hg higher than in the right atrium (*Fig. 4*). In mitral valvular disease, especially in stenosis combined with bicuspid insufficiency, the value of the gradient may amount to several times the normal (*Table I*, patient 4). Likewise, there was a conspicuous difference in oxygen saturation between the blood samples taken from the azygos vein and from the segment of the superior vena cava above its entrance. In the control group this difference did not exceed 5 per cent (1 vol per cent), whereas in patients 1 to 6 it was about 10 per cent (2 vol per cent).

In case 13 there had been multiple pulmonary embolisations in addition to combined mitral valvular disease during the 7 years of observation. The patient died suddenly of acute heart failure. The corroded preparation of the right lung demonstrated the experimental results of MILLER [4]. Through the two bronchial veins found in the hilum the pulmonary venous system of the middle lobe was perfectly filled up.

Summing up, our results allow for the following conclusions.

1. In pulmonary venous hypertension (e.g. in mitral disease) part of the blood is diverted from the system of the pulmonary vein through the bronchopulmonary shunts into the right atrium.

2. The increased rate of bronchopulmonary venous flow may be demonstrated by catheterisation of the azygos vein.

3. The increase of the bronchopulmonary shunt volume flowing in the direction of the right atrium augments the work of the right heart. Since it is especially in mitral disease that the shunt flow tends to increase, bronchopulmonary venous flow is also maintained by the overburdened right heart (pulmonary artery — pulmonary capillary — pulmonary vein — venous bronchopulmonary shunt — bronchial vein — azygos vein — superior vena cava — pulmonary artery etc.).

4. It seems likely that the bronchopulmonary shunt reduces the congestion in the pulmonary venous system.

5. An increase in arterial bronchopulmonary flow places a greater burden on the left than on the right heart [3] (aorta-bronchial artery — arterial bronchopulmonary shunt — pulmonary capillary — left atrium — left ventricle — aorta, etc.).

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EFFECT OF POTASSIUM PERCHLORATE ON THE FOETAL RABBIT THYROID

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The effect of protracted potassium perchlorate treatment from the beginning of pregnancy on the maternal and foetal thyroid glands has been studied in rabbits. The substance has been found to cause excessive hyperplasia and hyperfunction in the maternal and foetal gland alike. The increase in the relative weight of the foetal glands was more intensive than that of the maternal gland, a finding which is a sign of higher goitrogenic risks to the foetus and suggests that the foetal hypophyseothyroid system has functions of its own, independent from those of the maternal organism. Attention is drawn to the foetal risks involved by the treatment in question.

The adverse effects of intrauterine thyroid injury on physical and mental development have been widely discussed (BONGIOVANNI et al. [1956], FRENCH and VAN WYK [1964], HOOFT et al. [1962], JOB [1963], NEIMANN et al. [1963]). Iatrogenic factors account for a large number of such injuries. Potassium perchlorate treatment, claimed by its initiators (GODLEY and STANBURY [1954]) to be entirely harmless has been subsequently incriminated by others (GJEMDAL [1963], KRAATZ and MÄRZ [1960], KREVANS et al. [1962], SUNAR [1963], etc.) for goitrogenic and other effects. The increasing use of potassium perchlorate in the treatment of hyperthyroidism in recent years warrants investigations into its effects during pregnancy.

In earlier investigations we have found that thyroid ^{131}I uptake which is the first sign of the gland's activity (MITSKEVITCH and MAMULY [1953]) starts on the 20th day of foetal life in the rabbit (LAMPÉ et al. [1966a]). This is the stage when differentiation of the acini begins and the first traces of colloid appear. By the end of intrauterine life the microscopic or functional differences between the foetal and the maternal gland have disappeared, as reflected by identical histochemical reactions of both tissues (LAMPÉ et al. [1966b]).

Our investigations into the effect on the foetal thyroid of potassium perchlorate administered over the whole pregnancy were concerned with the following issues.

1. Do thyrostatic agents entering the foetal organism by the placentar route, induce similar changes in the foetal as in the maternal gland?

2. Do these agents interfere with the normal differentiation of the thyroid gland?

Material and method

12 pregnant rabbits kept under identical nutritional and environmental conditions were given potassium perchlorate in doses of 100 mg/kg daily, mixed to a small portion of food, from the very start of pregnancy. Six animals were worked up on the 21st day, six others on the 28th day of pregnancy, our earlier experiments having shown differentiation of the thyroid gland to start on the 20th and to complete its course by the 28th day of pregnancy. We examined the maternal thyroids in order to check the effect of the drug. As a control, data for untreated rabbit foetuses obtained in earlier experiments were used.

At the end of the respective periods the animals were killed, the foetal thyroids removed and worked up, together with the maternal thyroid and pituitary glands for histological and histochemical study and statistical evaluation. The glands were freed from the adjacent tissues as completely as possible, then weighed and related to 100 g body weight. For staining, combined gallocyanin-orcein-eosin-methanyl-yellow and the haematoxylin chromatrope 2R method were used, and as histochemical methods, the PAS-reaction for the demonstration of glycoproteins (neutral mucopolysaccharides); toluidine blue, celestine blue and Hale's reaction for the demonstration of basophilic elements; tetrazotised benzidine for the demonstration of protein; diazonium for the demonstration of SS + SH groups.

The reason for selecting the foregoing reactions was to have found in earlier studies, in accordance with KROMPECHER et al. (1961a, 1961b, 1962), MÜLLER (1962), KOBAYASHI et al. (1959) and WALLRAFF (1959) that the substance which gives the thyroid gland its histochemical character is a neutral glycoprotein, the carrier of the actual thyroid hormones. By identifying the carbohydrate and the protein components of this substance the reactions will consequently define the secretory state of the thyroid.

Statistical evaluation of the microscopic picture allowed a reliable estimation of thyroid function. Owing to the inhomogeneous structure of the thyroid gland, we found the quantitative procedure of ERÄNKÖ (1955) and PALKOVITS (1963) based on the measurement of the acinus–epithelium–stroma ratio, more suitable for this purpose than measurements of nucleic variae tions. The maternal and foetal thyroid glands of untreated animals belonging to the same stages of pregnancy were studied by the same method.

The conclusions drawn from the present experiments have been based on a comprehensive evaluation of the evidence derived from the microscopical, histochemical and statistical methods and of the thyroid weights.

Results

Fig. 1 shows the relative maternal and foetal thyroid weights. The thyroids of the untreated pregnant animals weighed between 5.0 and 11.4 mg/100 g [average, 8.1 mg/100 g] on the 21st day and between 7.7 and 13.3 mg/100 g [average, 10.45 mg/100 g] on the 28th day. The thyroids of the foetuses of the same animals weighed between 11.7 and 18.7 mg/100 g [average, 13.8 mg/100 g] on the 21st day, and between 11.3 and 13.9 mg/100 g [12.5 mg/100 g] on the 28th day.

Ingestion of perchlorate has been found to cause an increase in maternal as well as in foetal thyroid weight. On the 21st day of treatment the maternal thyroids weighed between 10.3 and 28.9 mg/100 g (average, 21.7 mg/100 g), and the foetal thyroids, between 42.3 and 56.2 mg/100 g (average, 49.5 mg/100 g). In other words, the maternal thyroid reached nearly threefold, and the foetal gland, nearly fourfold its initial weight. Continued intake of perchlorate further enhanced the increase in thyroid weight, particularly in the foetus. The maternal gland weighed between 28.3 and 51.1 mg/100 g (average, 41.0 mg/100 g), and the foetal gland, between 85.0 and 131.7 mg/100 g (average, 110.2 mg/100 g) on the 28th day.

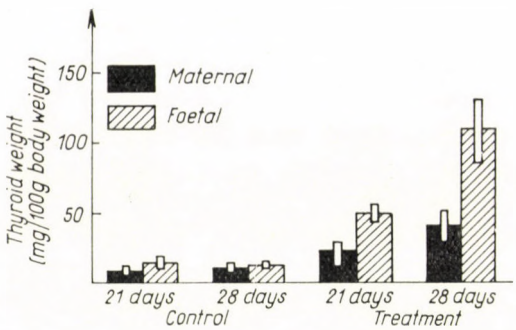
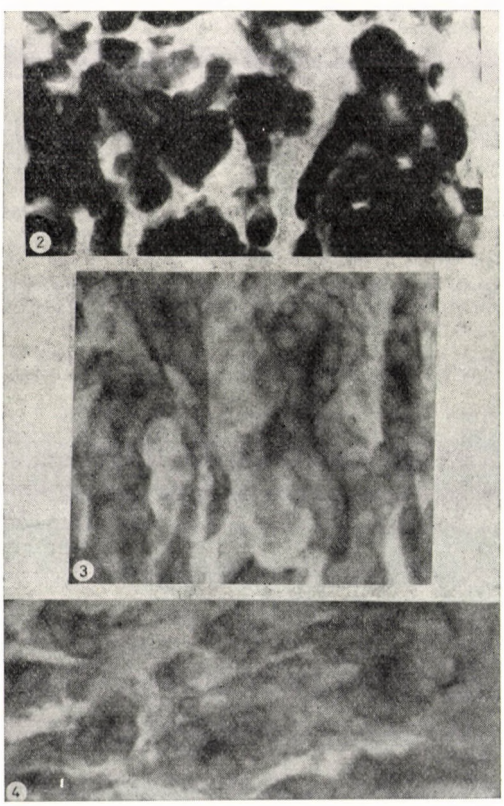


Fig. 1. Relative maternal and foetal thyroid weights in untreated rabbits and rabbits treated with potassium perchlorate, on the 21st and 28th days of pregnancy



Figs 2, 3, 4. Foetal rabbit thyroid on the 21st day of potassium perchlorate treatment continued from the beginning of pregnancy

Fig. 2. Glandular tissue consisting of dense trabeculae with minute acinar lumina. Gallocyanin-orcein-eosin-methanyl-yellow staining (GOEM). Enlargement: 40 × 1.5 × 4.1

Figs 3 and *4.* Thyroid gland of the same rabbit foetus. PAS-reaction. The acini are lined with high cuboidal and columnar epithelial cells. The minute lumina contain PAS-positive colloid. Enlargement: as before

Microscopical changes produced by $KClO_4$, during the first 21 days of pregnancy

Foetal thyroid. The pattern is made up of dense trabeculae and occasional acini. The epithelial cells are cuboid in shape and contain round nuclei with a loose chromatin structure. The lumina of the scarce acini not wider than a pinhole contain PAS-positive colloid (Figs 2, 3, 4). Apart from higher epithelial cells neither the microscopical nor the histochemical picture differs from that seen in untreated animals. Statistical evaluation reveals a reduction in the relative number of the acini to 4 per cent as against 10 per cent in the controls, while the relative volume of the stroma has been found to increase from 39 per cent to 46 per cent. The proportion of the epithelium remained practically unchanged (Fig. 5).

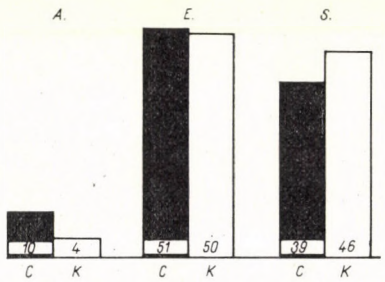
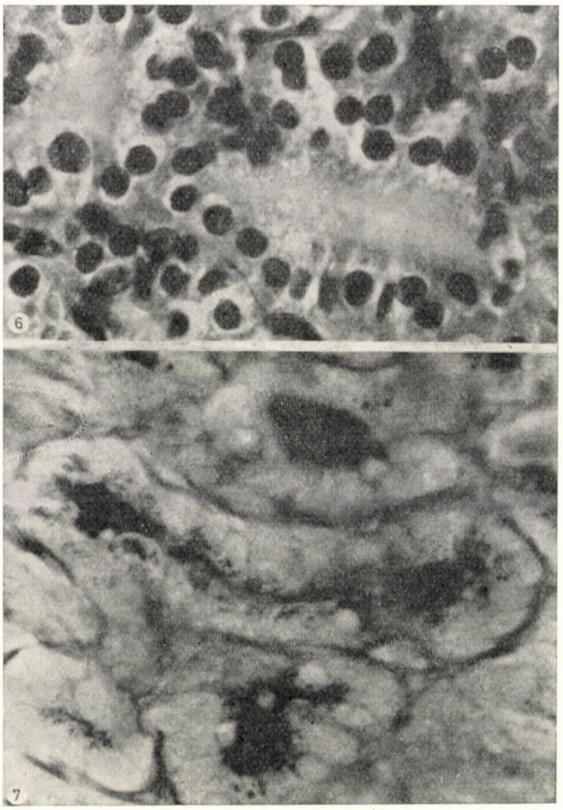


Fig. 5. Proportion of acini (A), epithelial cells (E) and stroma (S) in the foetal rabbit thyroid on the 21st day of potassium perchlorate treatment (K) and in untreated controls (C)

Maternal thyroid. The microscopic pattern is dominated by acini lined with a high columnar epithelium interspersed by cuboidal cells. The acini contain traces of PAS-positive colloid of a very loose, foamy structure. The apical zone of the columnar cells shows an excessive number of PAS-positive granules. The microscopic picture is thus characteristic of hyperfunction (Figs 6, 7). The distinctive change in relation to the maternal glands belonging to the same stage of pregnancy is hyperfunction. Statistical evaluation shows a reduction in the percentage of acini and a parallel increase in that of functioning epithelial cells with no quantitative change of the stroma (Fig. 8).

Ingestion of $KClO_4$ between the first and 28th day of pregnancy

Foetal thyroid. The greatly enlarged hyperplasic gland is made up of acini with a lining of columnar epithelium and very narrow, invariably colloid-free, lumina (Figs 9, 10). The absence of colloid deposits, the structural signs of epithelial hyperfunction and retarded development of the acini are in sharp contrast with the pattern found in the foetuses of untreated animals. Statistical evaluation shows a reduction in the relative number of acini and a parallel increase in that of the epithelial cells (Fig. 11).



Figs 6 and *7*. Thyroid gland of rabbit on the 21st day of pregnancy. Treatment with perchlorate from the first day of pregnancy

Fig. 6. Hyperfunctioning tissue. GOEM-staining. Enlargement: as on Fig. 2.

Fig. 7. PAS-positive colloid and granules in the apical zone of the epithelial cells. Enlargement: as above

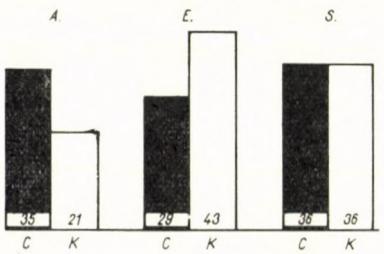
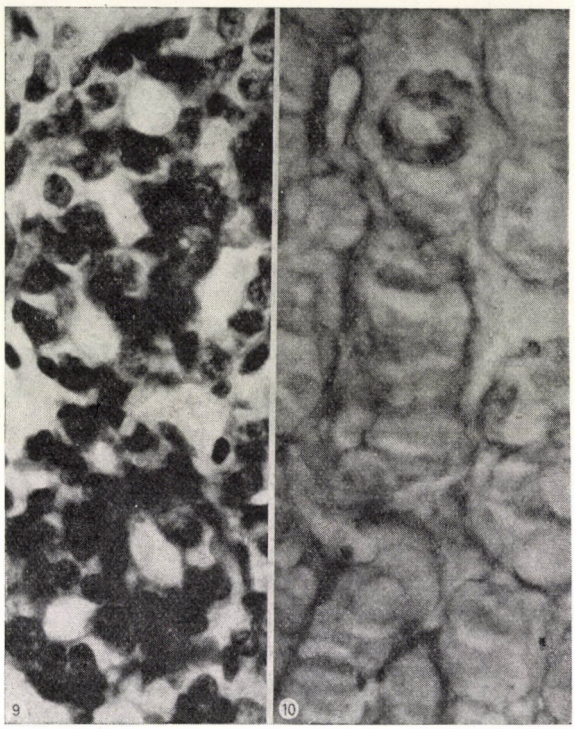


Fig. 8. A—E—S ratio in the maternal thyroid on the 21st day

Maternal thyroid. The acini are lined with columnar epithelium and contain PAS-positive, loose, sporadically dense, colloid (Figs 12, 13, 14, 15). The glandular hyperfunction is in sharp contrast with the glands of untreated animals being in the same stage of pregnancy. Statistical evaluation reveals



Figs 9 and *10.* Foetal thyroid on the 28th day; potassium perchlorate having been administered since the first day of pregnancy

Fig. 9. Differentiation of acini and dense trabeculae. No colloid deposits. High cuboidal glandular epithelium. GOEM-staining. Enlargement: as before

Fig. 10. PAS-reaction. The basement membrane and the apical membrane of the glandular epithelial cells are PAS positive. No colloid reaction. Enlargement: as before

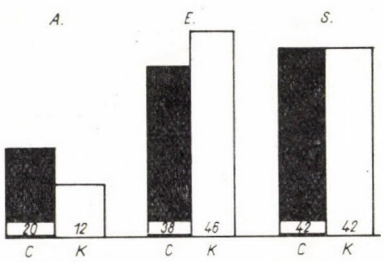
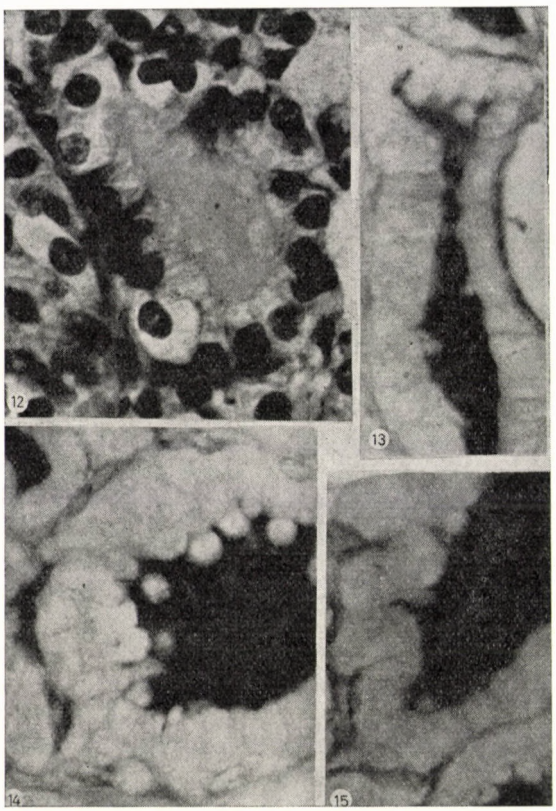


Fig. 11. Quantitative relation between A—E—S in the foetal gland on the 28th day

a reduction in the relative number of acini and an increased proportion of epithelial cells and stroma (Fig. 16).

Maternal hypophysis. Among the beta cells many vacuolated, typically degranulated "thyroidectomy-cells" are seen regardless whether treatment has been of 21 or 28 days duration.



Figs 12—15. Thyroid gland of rabbit on the 28th day of pregnancy. Treatment with potassium perchlorate from the first day of pregnancy.

Fig. 12. Excessive glandular hyperfunction. Acini lined with high columnar epithelium. Occasional round giant cells with loose clear plasma. GOEM-staining. Enlargement: as before

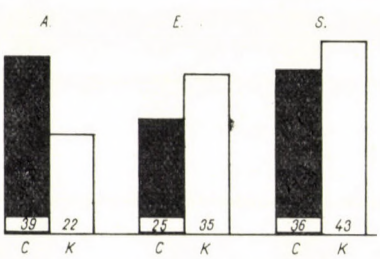


Fig. 16. Quantitative relation between A—E—S in the maternal thyroid on the 28th day

Discussion

The antithyroid activity of $KClO_4$ due to its thyroxine synthesis blocking effect has been shown by means of ^{131}I by WYNGAARDEN (1952) and confirmed morphologically by KRÜSKEMPER and KLEINSORG (1954).

The mechanism of iodine concentration by the thyroid, as the first step in the synthesis of thyroid hormone is claimed by LEWITUS et al. (1961), GRAB and OBERDISSE (1959) to be due to a binding of iodine by adsorption, a process in which the thyroid is supposed to play the role of a specific protein ion-exchanger. In opposition to this, HALMI et al. (1956, 1961) regard the accumulation of iodine as an energy-consuming active transfer process. Perchlorate inhibits the iodine-concentrating activity of the thyroid as well as in other systems in which iodine is deposited, i.e. in the salivary glands, in the gastric wall, etc. (WYNGAARDEN et al. [1953], HALMI et al. [1956], KUTZIM et al. [1963]). A comparative antagonism blocking the access of iodine to the thyroid is ascribed to $KClO_4$ by WYNGAARDEN, a claim consistent with the finding of ANBAR et al. (1959) that ^{36}Cl and ^{18}O labelled $KClO_4$ is stored in the thyroid. The fact that KRÜSKEMPER and REILICH (1959) have been able to neutralize the effect of $KClO_4$ by KJ administration also supports this theory.

In the light of the foregoing experimental evidence as well as of numerous clinical observations (LŐRINCZ and ANDOR [1956], SZÁNTÓ [1964]) the manner in which $KClO_4$ takes effect may be outlined as follows. The substance most certainly interferes with iodine uptake whether through a competitive antagonism or a blocking effect on the enzymes providing for the accumulation of iodine. The consequence is a reduced production of thyroid hormone and a fall of the blood hormone level. This in turn elicits an enhanced production of TSH. This results in enlargement and hyperplasia of the thyroid gland the extent of which is related to $KClO_4$-intake. Involvement of the pituitary gland by the process has been demonstrated by EGER et al. (1955) by the finding of thyroidectomy-cells associated with low thyroxine levels.

As suggested by our findings, the permeability of the placental barrier to $KClO_4$ enables this substance to cause the same changes in the foetal thyroid as in the maternal gland, namely intensive hyperplasia as a sign of hyperfunction, and on the evidence of statistical calculations, a relative reduction in the number of acini with an increase in epithelial elements and stroma, associated with a loss or complete disappearance of colloid. In untreated animals, the relative weights of maternal and foetal glands have been found practically identical at the given stages of pregnancy, whereas under the effect of $KClO_4$ a significant enlargement of the foetal thyroid is demonstrable on the 21st, still more so on the 28th day of pregnancy. This finding conclusively shows, in agreement with the results of NIKITOVITCH and KNOBIL (1955) and of POSTEL (1957) that the pituitary-thyroid system of the foetus functions independently of its maternal counterpart, otherwise enlargement of the maternal thyroid would be, obviously, more intense than that of the foetal gland. Increased sensitivity of the foetal thyroid may be due either to a deficient function of the enzymes responsible for iodine uptake or to an undue accumulation of perchlorate. Both possibilities require further study.

In evaluating our results, allowance must be made for the fact that the animals used in the study were healthy and euthyroid. In clinical practice, however, the reason for prescribing $KClO_4$ during pregnancy is usually maternal hyperthyroidism in which case abnormal function of the foetal thyroid is exceptional. Consequently, $KClO_4$, while normalizing the function of the hyperthyroid maternal gland, unduly depresses the activity of the euthyroid gland of the foetus.

It must, therefore, be kept in mind that potassium perchlorate intake during pregnancy causes similar structural changes in the maternal and foetal thyroid and carries more severe goitrogenic risks to the foetus.

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DISTURBANCES OF CARBOHYDRATE METABOLISM IN INDUCED INFARCTOID CARDIOPATHY

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Carbohydrate metabolism of the heart in cardiopathy brought about by a cardiopathogenic diet has been studied in isolated heart preparations. In the dietary group glucose uptake was normal, lactic acid release was reduced and pyruvic acid release increased. The factor held responsible for the metabolic changes is a reduction in lactate dehydrogenase activity which supposedly affects the SZENTGYÖRGYI—KREBS cycle closely involved in mitochondrial function.

In earlier studies Sós et al. have shown that infarctoid changes appear in rats [12, 13], dogs [14] and cocks [14] fed a semisynthetic diet rich in cholesterol, vitamin D_2, sodium, chloride, phosphate, and calcium, but poor in potassium and magnesium. The animals developed hypertension and coronary sclerosis leading mostly to spontaneous cardiac death, ensuing in rats between the 5th and 6th, in cocks between the 14th and 20th week. The microscopic changes were round cell infiltration, coronary sclerosis with degenerative changes, and scars, fibrosis and metastatic calcification resulting from myocardial infarctions. Aortic plaques were also present. Coronary occlusion was seen in a few cases only.

The failure of inducing infarctoid changes in any reliable manner by experimental atherosclerosis alone as well as the finding that myocardial necrosis was not necessarily associated with coronary occlusion, were suggestive of a direct disturbance of myocardial metabolism by the diet. To clear this issue was the aim of the present study.

Materials and methods

In the experiments 115 male rats of the Institute's stock were used. Forty animals serving as controls received a normal diet [13], 75 animals the cardiopathogenic S_{65} diet. For details of the diet see [13]. Both diets were semisynthetic. In the 5th and 6th week the hearts were isolated in both groups by the method of LANGENDORFF as modified by BLEEHEN and FISCHER [2] and perfused for half an hour with a Krebs—Henseleit bicarbonate Ringer solution containing half the amount of calcium [11] and 100 mg per 100 ml of glucose. The animals were fasted for 16 hrs before the experiment, then they were given 50 U/100 g of heparin intraperitoneally. Fifteen minutes later the animals were decapitated, the heart was suspended immediately on a cannula and perfused with 10 to 15 ml oxygenated fluid of 30 ± 0.05 °C under a pressure of 120 cm water. After 30 min. perfusion was stopped, the heart was carefully blotted, weighed to the accuracy of 10 mg and prepared for histologic study. The perfusion fluid was measured to the accuracy of 0.1 ml and stored in a refrigerator at $+2$ °C

after having added 50 mg NaF for the prevention of glycolysis. In aliquots of the perfusion fluid, glucose was estimated according to HULTMAN [10], pyruvic acid according to FRIEDEMANN and HAUGEN [4], lactic acid according to BARKER and SUMMERSON [1], Na and K by flame photometry, always on the day of the experiment. The uptake of glucose as well as the release of pyruvic and lactic acids were expressed in mg per 1 g wet heart weight per 1 hr. Blood pressure was measured indirectly [5]. Significance was evaluated by STUDENT's t-test. The sections were stained with haematoxylin-eosin.

Results

The animals weighed between 80 and 100 g at the outset. At the end of the experiment average body weight had risen to 143.7 g in the controls and remained at 97.4 g in the S_{65} group. Blood pressure was 110 $\pm$ 5 mm Hg in the controls and 160 $\pm$ 10 mm Hg in the S_{65} group. These figures agree with earlier findings [13].

The difficulties involved by the technique of heart preparation caused us to restrict perfusion to 30 min, this having been found the utmost period during which even the damaged hearts were functioning normally. This was the case in 40 out of 45 control hearts as opposed to 38 out of 70 hearts of the S_{65} group, a difference which alone would be conclusive of the adverse effect on the heart of the dietary factor. The remaining 32 hearts of the S_{65} group, 5 to 10 minutes after insertion of the cannula showed ventricular fibrillation followed by cardiac arrest. A number of control hearts continued to beat normally for one to three hours. In the S_{65} group only those hearts were evaluated which had functioned normally for 30 minutes.

The results are shown in Table I.

Table I

	Control group	Cardiopathogenic group	Significance
Wet heart weight per 100 g body weight	0.343 $\pm$0.170* (40**)	0.361 $\pm$0.167 (40)	$0.7<P<0.8$
Glucose uptake mg per g heart weight per hour	8.49 $\pm$2.79 (18)	8.09 $\pm$2.13 (18)	$0.8<P<0.9$
Lactic acid production mg per g wet heart weight per hour	2.94 $\pm$1.06 (17)	1.14 $\pm$0.72 (18)	$0.02<P<0.05$
Pyruvic acid production mg per g wet heart weight per hour	169.8 $\pm$54.8 (20)	345 $\pm$50.67 (19)	$0.01<P<0.02$

* = standard deviation
** = number of animals

Heart weight correlated to body weight showed no significant difference between the two groups. This indicates that the abnormal findings should be connected with functional causes rather than with cardiac hypertrophy.

Average glucose uptake was insignificantly reduced in the S_{65} group.

Release of lactic acid was reduced to 39 per cent of the control value in the S_{65} group, the difference being highly significant. Conversely, the release of pyruvic acid in the S_{65} group was twice that of the controls, the difference being highly significant.

At the end of the experiment, the K and Na concentrations of the perfusion fluid were similar in the S_{65} group and the control group and differed from the Krebs solution by at most 5 per cent.

No correlations were found between blood pressure values, histological picture and the metabolic changes. In contrast, the life span of the isolated heart preparation and the microscopic changes were closely correlated. Severely damaged hearts rarely functioned after half an hour. In this manner, our observations were generally derived from less severely affected hearts; this, however, adds to the significance of the differences.

The microscopic findings were consistent with those described in the introduction.

Discussion

The significance of electrolyte metabolism in the changes under study has been confirmed by earlier investigations [12, 13, 14]. The present data direct attention to the involvement of carbohydrate metabolism. From the normal glucose uptake it may be inferred that glucose permeability and hexokinase (glucokinase) activity were unaffected and that the first steps of glycolysis proceeded normally. The heart affected by the S_{65} diet did not release or take up sodium and potassium, in other words, the electrolyte metabolism was permanently disturbed.

Pyruvic and lactic acid production was affected in a characteristic manner. The change was, however, the opposite of what we had expected to find in the presence of infarctoid lesions, particularly because there is evidence that in myocardial anoxia both the heart muscle and the venous blood leaving the heart contain reduced levels of pyruvic and elevated levels of lactic acid [3, 7]. The reduced lactic acid production in dietary myocardial injury may be ascribed either to an inhibition of lactic acid dehydrogenase, or to a fall in the concentration of reduced pyridine nucleotides ($NADH_2$). GUDBJARNASON [8] found in chronic heart failure reduced aldolase, lactic dehydrogenase and NADP-isocitrate dehydrogenase activities, with a simultaneous increase in the activity of glycerol aldehyde phosphate dehydrogenase as a sign of aerobic compensation for the disturbance of the anaerobic metabolism. On these grounds the reduced production of lactic acid might be best accounted for by an inhibition of lactate dehydrogenase. It is hoped that direct measurements will clarify these questions including the role of $NADH_2$.

Accumulation of pyruvic acid may have its cause, apart from disturbances of the SZENTGYÖRGYI—KREBS cycle, or of aerobic metabolism in general, also in thiamine deficiency. This is supported by the finding of high blood pyruvic acid levels and a reduced cardiac pyruvic acid extraction in dogs kept on a thiamine-deficient diet [9]. It has been also possible to inhibit oxidation of pyruvic and of α-ketoglutaric acids in brain and liver cell mitochondria by the administration of thiamine antagonists [6], a finding which incidentally explains the cardiac changes in beri-beri. The fact that the S_{65}-diet contains sufficient amounts of vitamin B_1 suggests that the citrate cycle is involved in consequence of anoxia, but it is well possible that the diet leads to a relative B_1-hypovitaminosis by increasing the demand for thiamine. In sum, the metabolic changes brought about by the S_{65} diet are to a certain degree reminiscent of those found in congestive heart failure, and it is assumed that myocardial metabolism is directly affected and not secondarily through hypoxia resulting from atherosclerosis. The dietary injury seems to involve primarily the mitochondria, since it is there that pyrivuc acid decarboxylation takes place and where the enzymes of the SZENTGYÖRGYI—KREBS cycle are to be found.

It is an open question whether the electrolyte disturbances revealed in earlier studies [12] represent primary changes or whether they result from altered cell functions. The lipid and protein metabolism of the heart in the presence of infarctoid lesions is also a subject for further studies.

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PRESSURE MEASUREMENTS IN VARIOUS PARTS OF THE LYMPHATIC SYSTEM

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In 23 anaesthetized dogs pressure has been measured in different parts of the lymphatic system, i.e. on the neck (thoracic duct, jugular trunk, right lymphatic trunk), in the chest (efferent lymph vessel of the heart, bronchomediastinal trunk), in the abdominal cavity (hepatic trunk, intestinal trunk, lumbar trunk). The results lend further support to the claim that centripetal lymph flow is maintained by temporarily acting regional forces rather than by a constant pressure gradient.

In a previous paper [1] it has been shown that neither the pressure differences between the thoracic duct and the cervical veins, nor those between the thoracic duct and peripheral — i.e. hepatic, lumbar, intestinal, leg — lymph vessels provide a constant gradient which might possibly account for the maintenance of lymph flow. It must, however, be stated that very little is known about the hydrostatic pressure prevailing in the various areas of the lymphatic system, including its main trunks. Pertaining data in the literature are not comparable in view of the different techniques employed. This has prompted us to study the pressures in different regions of the lymphatic system.

Methods

Mongrel dogs under chloralose general anaesthesia (0.1 g/kg) were fixed in the supine position to the operating table. On the left side of the neck, the thoracic duct and the jugular trunk, and on the right side, the right lymphatic trunk or the right jugular trunk were exposed. The former proved to be a duct 1 to 2 cm long, emptying into the right venous angle. In many animals there was no actual right lymphatic trunk, but the individual ducts making up the trunk joined separately the cervical veins. In this case pressure was measured in the lymph duct with the greatest calibre just over its junction with the vein. Pressure in the jugular trunk was usually measured at the left side at least 3 to 4 cm above its junction with the thoracic duct. In a few cases, pressure was measured bilaterally. Then the chest was opened and under intratracheal mechanical breathing the bronchomediastinal trunk and the efferent lymphatic vessel of the heart [2] were exposed and their pressure measured. Closure of the chest was followed by upper median laparotomy and the main trunks carrying lymph from the liver, the intestinal and lumbal (generally the left one because of easier access) trunks were exposed and the pressures were measured. Occasionally, pressure was determined in the right lumbal trunk and in the lymphatics of the perirenal capsule and the renal hilum, or in other cases, in one of the lymphatics running parallel with the saphenous vein. For the measurements a Statham type strain gauge fitted to a Siemens electromanometer was used; the zero point of the manometer was adjusted 2 to 3 cm above the plane of the table.

A French 1 (No. 30) cannula was inserted retrogradely into the lymph duct and connected with the manometer's measuring-head by means of a thin, non-elastic teflon tube filled with

Table I

Intralymphatic pressures

No.	Jugular vein	Thoracic duct	Right lymphatic trunk	Jugular trunk (left)	Heart	Bronchomed. trunk
1.	6.5	5.5		0.2		
2.	5.0	6.0		0.5		
3.	7.5	6.5		0.3		
4.	8.5	9.0		0.2		
5.	7.5	7.0	2.0	0.7	5.0	3.0
6.	4.5	3.0	3.5	1.0	2.0	2.0
7.	7.0	5.0	4.0	3.2	2.0	5.2
8.	10.0	10.0	1.8	0.5	3.5	3.0
9.	8.0	6.5	1.0	0.2	4.5	
10.	4.0	3.5	—1.0	—0.5	4.0	3.5
11.	8.0	5.0		3.5		
12.	9.0	8.0	7.0	5.5		
13.	5.0	3.0	2.0	0.3	1.5	
14.	2.0	2.0	1.0	0	1.0	2.0
15.	3.0	10.0	1.0	0.2	2.0	0.5
16.	4.0	2.0	3.0	0	3.0	1.0
17.	7.5	1.0	3.0	—1.0	2.0	1.5
18.	6.0	4.0	1.0	0.5	2.0	1.0
19.	1.0	2.0	1.5	0.2	5.0	
20.	6.5	4.0	1.5	0.5	3.5	1.5
21.	7.0	5.0	—0.8	0.2	3.5	1.5
22.	3.5	7.0	2.0	0.8	4.5	
23.	3.5	3.5		1.0	0.5	1.5
Mean	5.83	5.11	2.13	0.85	2.91	2.09
	1.0—10.0	1.0—10.0	—1.0—7.0	—1.0—5.5	0.5—5.0	0.5 —5.2
S	0.40	0.46	0.40	0.28	0.34	0.35
n	23	23	17	23	17	13

heparin solution. Care was taken to avoid obstruction of the lymph flow with the cannula. This was namely occasionally observed in our earlier experiments and at the beginning of the present studies as long as we had been directing the tip of the cannula proximally which led sometimes to erroneously low readings by obstructing lymph flow, especially in small-calibre vessels. When, however, the tip of the cannula points distally, that is in the direction opposite to the lymph flow, then obstruction will lead to a successively rising pressure which the instrument will not fail to disclose.

As stated in an earlier study [1], the non-integrated pressure curves exhibit oscillations. In the present experiments, mean pressure values were recorded by electronic integration.

(mm Hg)

Liver	Intestinal trunk	Lumbar trunk (left)	Perirenal capsule	Renal hilum	Leg
4.5	8.0	2.0			0.0
2.0	6.0	2.2			1.0
3.8	3.5	1.7			
7.5	8.5	2.5			−0.3
					−0.3
					2.7
					0.0
2.0	6.0	7.0			
2.0	2.5	2.0			
3.0	1.5	4.0			
3.5	0.5	1.5			
0.3	1.5	1.0			
1.5	0.3	1.5	1.0	6.0	1.0
8.5	4.0	2.0	5.0		
3.5	3.5	5.0			
2.5	1.0	3.0	9.5	5.0	0.0
3.43	3.60	2.72			0.51
0.3—8.5	0.3 —8.5	1.0—7.0	1.0—9.5	5.0—6.0	−0.3—+2.7
0.64	0.77	0.28			0.38
13	13	13	3	2	8

Results

A total of 23 animals was used. The pressure values are shown in Table I. Mean pressure in the jugular vein was 5.83 mm Hg; in the thoracic duct, 5.11; in the jugular trunk, 0.85; in the right lymphatic trunk, 2.13 mm Hg. With the chest open, pressure in the efferent lymphatic trunk of the heart was 2.91 mm Hg; in the bronchomediastinal trunk, 2.09 mm Hg; in the hepatic trunk, 3.43;

in the intestinal trunk, 3.60; in the lumbar trunk, 2.72 mm Hg; in the crural lymph vessel, 0.51 mm Hg.

Pressure in the lymphatics of the perirenal capsule, measured in three instances was found unexpectedly high, i.e. from 1.5 to 9.5 mm Hg. Pressure in the renal hilum, measured in two cases, was 5.0 and 6.0 mm Hg, respectively. Measurement of pressure in the renal lymphatics was attempted only if clearly visible vessels allowing cannulation could be found. This was, however, an exceptional finding, due probably to congestion. Between the pressure values of the two lumbar trunks no significant difference was found. In four cases simultaneous measurements were made on both sides, the readings were 2.1 (1.0 — 3.0) mm Hg right and 2.3 (1.0 — 4.5) mm Hg left. In parallel readings performed on both sides in six cases the pressure in the right jugular trunk was 0.33 ± 0.13 and in the left, 1.08 ± 0.58 mm Hg. The difference between the two sides was, however, not significant (one-sample "t" test; $p = 0.30$).

Discussion

Confrontation of the present findings with the sparse data recorded in the literature shows that the pressure found in the thoracic duct by ROUVIÈRE and VALETTE [4], i.e. 6.4 cm H_2O, and by WEBB and STARZL [5], i.e. 3.5 to 5.5 cm H_2O are inside our limit values (1.0 to 10.0 mm Hg). The mean pressure recorded by PAPP [6] in the thoracic duct, 2.5 (-2.0 — $+7$) cm H_2O was remarkably low. MCCARRELL [7] registered pressures between -2.8 and $+3.2$ cm H_2O in the jugular trunk during passive movement of the head, whereas ROUVIÈRE and VALETTE obtained figures between 1.5 and 2.0 cm H_2O and WEBB and STARZL between 2.5 and 10.0 cm H_2O by indirect measurements. The values found in the present study ranged between -1.0 and $+5.5$ mm Hg. Values in the order of those recorded by WEBB and STARZL have never been found in our cases except in systemic venous congestion [1], in which case the pressures in the jugular trunk ranged between 3.0 and 14.5 mm Hg.

In respect to the lymphatics of the heart, the figures given by DRINKER et al. [8] refer to end pressures, the maximum value being 15.5 cm H_2O. These are, obviously, not comparable with our value of 2.91 ± 0.34 mm Hg since the end pressure greatly exceeds the side pressure in the lymphatics. We found for instance a mean end pressure of 26.3 (16—32) cm H_2O in the thoracic duct of 10 healthy dogs as opposed to the side pressure of 5.11 ± 0.46 recorded in the present experiments.

In the lymphatics of the legs, DRINKER and FIELD [9] never found positive resting values, in fact, the resting pressures ranged around zero. This is in agreement with our own findings. Flexion and extension alternating in rapid succession result in a rise of side pressure to 58 cm H_2O, and this figure further rose to 99 cm H_2O when the lymphatics had been compressed.

By the observation that in the lymphatics of a resting limb, there is virtually no measurable pressure it is convincingly illustrated that in the lymphatic system there is no *vis a tergo*, in other words, no intrinsic pressure gradient which would maintain a continuous centripetal lymph flow. Correspondingly, as pointed out by GENERSICH as early as 1871 [10] the resting limb has no lymph flow either. In the same way, the outflow of lymph from the cervical trunk of the resting animal is scanty or nil. It requires massage of the neck or passive motion of the head to elicit lymph flow [7]. The local factors of lymph flow have been discussed in another study [11, 12]. The factors responsible for the maintenance of intralymphatic pressure have also been dealt with [1]. In the present study the side pressures were measured in various areas of the lymphatic system. By the use of the same technique in identical animals comparable figures have been obtained which plead against the existence of a stable pressure gradient between the central and the peripheral parts of the lymphatic system sufficient to maintain the centripetal flow of lymph.

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EFFECT OF TOTAL BODY IRRADIATION ON CAPILLARY PERMEABILITY

FLUID AND ELECTROLYTE BALANCE, AND INTRAORGANIC PLASMA PROTEIN SPACES

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Total body irradiation by 650 r was followed in the rat by a reduction in plasma protein level as well as in the dry weight and protein-N concentration of the individual organs. Plasma Na and K levels were unaffected while Na concentration was increased in the spleen and in the skin, reduced in the intestinal wall, and the K concentration was in most tissues reduced.

The intravascular albumin space determined three minutes after intravenous administration of ^{131}I-albumin revealed a general increase in the irradiated animals, being significant only in the kidney and spleen. Total intraorganic albumin space determined 24 hrs. after administration of the labelled albumin showed no increase in any individual organ. There was a conspicuous finding of identical intravascular and total intraorganic albumin spaces, i.e. complete disappearance of extravascular albumin, in the liver. Analysis of the results shows that irradiation causes no excess of extravascular protein, and the fluid excess in the tissues is not extracellular, i.e. oedematous in character, no correlation having been detected between the intra- and extravascular fluid and electrolyte changes. The present results thus leave any significant involvement of capillary permeability out of consideration.

Capillary changes caused by ionizing radiation are generally described as fairly characteristic [31]. Capillary dilatation is produced, this appears as cutaneous erythema. From the observation that colloidal dyes injected intravenously reach the affected areas rapidly [21] and that they are cleared from the circulation at an increased rate [11, 35], it had been concluded to an increase in capillary permeability. Later studies, however, indicated that it is not capillary permeability which is at fault but rather the return of colloids, including plasma proteins, from the extravascular space by the lymphatics into the blood [28, 29]. Both possibilities imply an increase in the amount of extravascular protein which may result in a reduction of plasma volume and/or in a loss of plasma proteins on the one hand and in an increase in extracellular fluid volume, on the other — i.e. in oedema — as reflected by an increase in tissue fluid and sodium content associated with a decrease in the concentration of potassium. Obvious as this process seems to be, the pertaining data are conflicting. Some authors regard the increase of plasma volume as a mechanism compensating the destruction of red blood cells [14, 22, 24, 26], while others found plasma volume to be reduced [10, 29, 33]. At any rate, the dose of radiation and the stage at which the changes are being studied seem to make a great difference [26]. The plasma protein level was, however, consistently diminished

in all of the animal species hitherto studied [15, 24, 29]. Again, some authors found a slight increase in the volume of extracellular fluid whereas others observed next to no change at least as far as the acute stage is concerned [24, 34].

Evidence is scarce concerning the electrolyte concentrations in the tissues. Though studies *in vitro* seem to suggest that ionizing radiation elicits a loss of potassium and an uptake of sodium by the cells, more particularly by the erythrocytes [16], numerous studies in various animal species have failed to demonstrate any significant change in the plasma sodium and potassium levels [3, 4, 30, 24], though there were occasional findings to the contrary.

We have thus no reliable information on the question whether lethal total body irradiation causes an increase in capillary permeability, as reflected by a general or local oedema, nor on the question of particularly affected tissues or systems. To clear these issues we have studied the effect of irradiation in the rat on blood plasma volume and protein level, on the fluid, protein and electrolyte content of individual tissues, furthermore on the intra- and extravascular plasma protein space.

Methods

Forty female albino rats weighing 267 $\pm$ 4 g were used. Another group of 40 animals weighing 264 $\pm$ 6 g served as controls. The animals received a total body irradiation of 650 r with a Siemens Stabilivolt apparatus, with 180 kV, 15 cm mA, 1 mm Cu filter, skin—focus distance 80 cm. This dose almost infallibly caused death of the animals by the end of two to four weeks. Our studies were performed as soon as the radiation syndrome had developed. As its indicator the red cell count was used, this having been found more reliable for this purpose than the leukocyte or lymphocyte counts. At the start of the studies the initial red cell count of 6.086,000 $\pm$ 111,000 had fallen to 2.562,000 $\pm$ 101,000, whereas the initial leukocyte count 13,560 $\pm$ 692 fell to 3760 $\pm$ 336. On the basis of these criteria the studies were performed between the 8th and 22nd day (average 12.7 days). In consequence of the radiation injury the animals showed a loss of weight amounting to 12.4 $\pm$ 1.3 per cent.

At this stage, the animals received into the caudal vein 0.1 ml/100 g of ^{131}I-labelled human serum albumin with an activity of 4 to 6 μC. Then the animals were studied in two groups of 20 rats each. In the first, blood was drawn from the carotid three minutes after the injection of labelled protein and the animals were killed by an overdose of the anaesthetic, in the second group this procedure took place 24 hrs. after the injection. The two non-irradiated control groups of 20 rats were studied in the same manner.

In the biochemical methods applied freezing techniques were avoided in view of the excessive scatter of the results which could not be eliminated even by triplicate determinations. The technique employed provided for more reproducible figures. After killing the animals the organs were left to bleed, then were cut up into pieces in the container used for further processing so as to avoid any further loss of tissue fluid. In each organ we determined the solid contents by drying until constant weight, and the concentration of protein-N by digestion with sulphuric acid and distillation according to WAGNER—PARNASS; the non-protein-N value was deduced from the obtained figure. Tissue specimens were homogenized in a Potter-homogenizer and extracted with trichloroacetic acid. The extracts served for estimation of Na and K (by flame photometry), of non-protein bound ^{131}I activity and N content. The same studies were carried out in the blood plasma. The total activity of plasma and of the specimens which had been digested with 20 per cent NaOH solution was measured in a scintillation well detector. After deducing the non-protein bound activity the quotients of the organ/plasma activities are equivalent with the albumin space in the respective tissue expressed as per cents of the wet weight. It was presumed that mixing for three minutes was sufficient for reliably reflecting the amount of intravascular plasma protein, whereas the distribution space obtained by the

end of 24 hrs. corresponded to the total intraorganic plasma albumin space, the difference between the two values giving the extravascular protein space.

The assumption that the reduction of the solid contents in the individual tissues, being the consequence of water uptake, allowed to estimate the volume of accumulated water per 100 g tissue, on the basis of the formula, $100 \cdot \frac{Mk}{Mr} - 100$, Mk representing the concentration of solids in the respective tissue of the control animal, and Mr in that of the irradiated animal.

Results

Circulating plasma volume was 4.30 ± 0.55 ml/100 g body weight in the controls, and 5.15 ± 0.56 ml/100 g body weight in the irradiated animals, as calculated from the plasma activities at three minutes. The difference did not

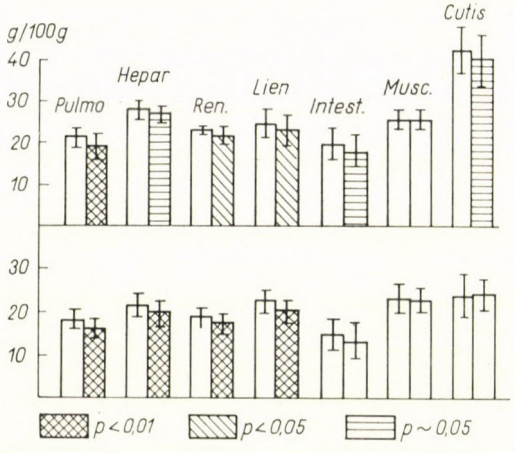

Fig. 1. Effect of total body irradiation on dry weight (upper row) and protein concentration (lower row) of the individual organs in rats. Left columns: control animals. Right columns: irradiated animals

reach significance ($p > 0.10$). Plasma protein concentration was 6.74 ± 0.11 g per 100 ml in the controls, and 5.69 ± 0.12 g per 100 ml in the irradiated animals. The difference was highly significant.

The solid content of the individual organs in the irradiated animals was generally reduced. Reduction in protein nitrogen content was highly significant ($p < 0.001 - p < 0.01$) in the lungs, the liver, kidneys and spleen, and not significant in skin, intestines and muscles. The total solid content was significantly reduced in the lungs, the kidneys, and the spleen, and it was at the borderline of significance in the liver, the intestines, and the skin. There was no reduction in the muscles (Fig. 1).

These figures, though being on the whole indicative of a fluid excess in the tissues are alone not conclusive of the presence of oedema. Apart from the role of cell breakdown confirmed by the considerable loss in body weight, water retention may be intracellular as well as extracellular. To obtain information on

this point, sodium and potassium concentrations in the tissues were studied.

Plasma Na and K levels were identical in the irradiated animals and in the controls, as Na was 127 ± 2, and 128 ± mEq/l and K 5.05 ± 0.15 and 5.3 ± 0.19 mEq/l, respectively. The changes of tissue Na concentrations were

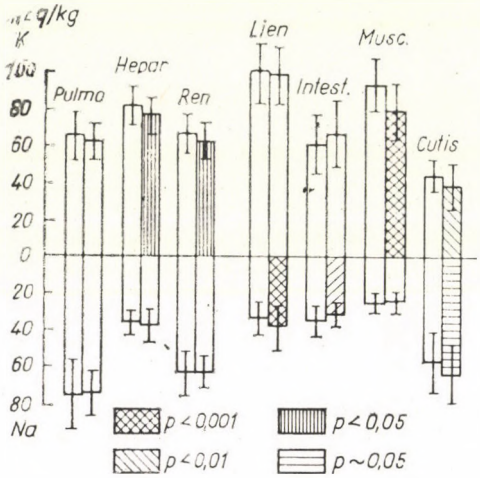

Fig. 2. Effect of total body irradiation on K and Na concentration of the tissues (mEq/kg wet weight). Upward: K-concentration. Downward: Na-concentration. Left columns: control animals. Right columns: irradiated animals

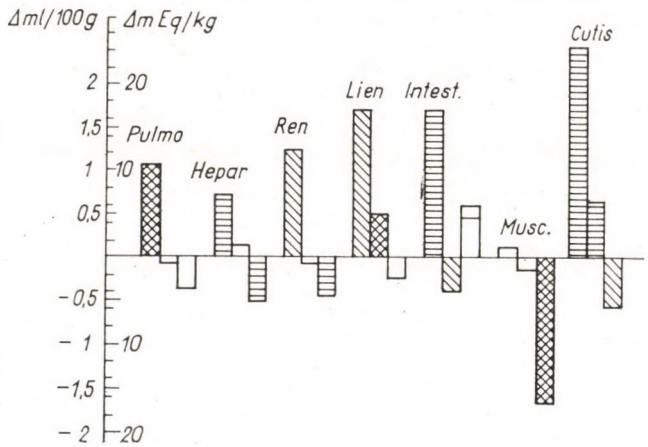

Fig. 3. Tissular fluid spaces and electrolyte concentrations in irradiated rats. Columns 1: Fluid volume (ml/100 g wet weight). Columns 2: Na concentration (mEq/kg wet weight). Left columns: control animals. Right columns: irradiated animals

not consistent; there was no significant change in the lungs, liver, kidneys, and muscles, but a significant increase was observed in spleen and skin, and a reduction in the intestinal wall. K concentration showed a parallel tendency to decline with the exception of the intestine where a not significant ($p > 0.10$)

increase was demonstrable. The reduction in the liver, kidneys, muscles, and spleen was, however, significant (Figs 2, 3).

The study of the intraorganic plasma protein spaces revealed a considerable increase in the irradiated animals at three minutes reflecting an increase in intravascular protein spaces. The increase in the kidneys and spleen was highly significant, whereas in the other organs it was not significant. The excess intravascular protein may be interpreted as having been due to vasodilatation or congestion. In contrast, neither the 24-hr. ^{131}I-protein space, i.e. the total intraorganic protein space, nor the extravascular plasma protein space, i.e. the difference of the 24-hr. and 3-minute values, revealed a significant increase in any of the individual organs. It was surprising to find a reduction of the total intraorganic albumin space in the liver, which was at the borderline of significance ($p > 0.05$, $p > 0.10$) and represented practically a total depletion of the organ of extravascular protein (Table I).

Table I

3-minute and 24-hour ^{131}I-albumin spaces [ml/100 g wet weight] in controls and irradiated rats

			Lung	Liver	Kidney	Spleen	Intestines	Muscle	Skin
Normal	3 min.	$\bar{x}$	11.4	12.6	14.0	7.1	1.3	0.42	0.94
		Sm ±	0.87	0.71	0.78	0.45	0.09	0.05	0.13
	24 hrs.	$\bar{x}$	23.7	18.1	21.4	12.6	9.4	3.3	7.70
		Sm ±	0.88	1.48	1.40	1.13	0.69	0.38	0.92
Irradiated	3 min.	$\bar{x}$	12.1	14.4	16.0*	9.0*	1.5	0.57	1.33
		Sm ±	0.77	1.40	0.70	0.61	0.15	0.06	0.20
	24 hrs.	$\bar{x}$	24.5	13.0	22.9	12.1	9.2	2.9	9.90
		Sm ±	1.62	0.57	0.94	1.59	0.80	0.23	1.03

* = $p < 0.01$

Discussion

Evaluation of the results from the point of view of capillary permeability makes it necessary to realize that the reduction in plasma protein concentration after radiation injury may be due to several factors, particularly to increased protein catabolism or loss, as well as to reduced anabolism, furthermore to a dilution of blood plasma. Passage of intravascular proteins into the extravascular space is presumably a factor of minor significance.

The discussion of the role of increased catabolism, reduced anabolism and of intestinal or renal protein loss, would not be justified in the present paper,

since these were outside the subject of the studies. As to the possible part played by dilution, this factor cannot be ruled out in view of the (not significant) increase in circulating plasma volume, in the present experiments. However in earlier studies on dogs [28, 29] a reduction was observed. Still, this reduction was proportionate to the loss in body weight, thus the ratio plasma volume per body weight had remained unchanged.

The present results did not support the occurrence of an excessive passage of proteins into the extravascular space. Though the concentration of ^{131}I-labelled albumin in the individual organs at three minutes, i.e. the intravascular plasma protein space, was slightly increased, there was practically no change in the total intraorganic plasma protein space as reflected by the 24-hr concentration of ^{131}I-protein in the individual organs in relation to the plasma protein level.

Some problems involved by our methods and the interpretation of our results should be, however, briefly discussed.

It is well known that the principle of indicator dilutions lends itself with certain limitations only for the estimation of circulating plasma volume. The results are reliable only when the indicator had completely mixed with the whole of plasma and did not leave the vascular bed in any significant amount.

The average mixing time of ^{131}I-labelled albumin is three minutes in the rat [7, 23]. This time is too short for any significant passage of labelled protein into the extravascular space (this problem will be discussed later). It must therefore be assumed that the total amount of ^{131}I protein is still inside the vascular bed, more particularly, as a consequence of our technique, inside the small vessels. Accordingly the indicator concentration found in a given tissue divided by the indicator concentration in the plasma gives the volume of intravascular plasma in the respective tissue. More than half the plasma protein of the body is however outside the vascular bed [1, 5, 17, 18]. In this manner, after intravenous administration of labelled protein an initial rapid intravascular mixing phase three to twenty minutes in duration, depending on the animal species, is followed by a further decline of the indicator concentration in plasma. During this slower phase, equilibration of the indicator with the extravascular plasma proteins is achieved. When this process is completed, the calculated volume no longer corresponds to that of the circulating plasma but to a fictive space in which the concentration of the respective proteins is the same as in the blood plasma. This space is a fictive one because the concentration of protein is no part of the extracellular fluid as high as in the plasma. It would therefore be more correct to express the amount of the respective plasma protein fraction (albumin in the present case) in the tissues in grams. This could be computed from the product of the "space" and the plasma concentration of the respective protein. In the present studies this has been avoided because in irradiated animals the plasma protein levels are decreased, therefore the calcu-

lations would not have reflected the true conditions. We found it more correct to indicate the total intraorganic plasma protein space or, more correctly, the intraorganic plasma albumin space. This, however, is not a reliable reflection of the amount of intraorganic plasma protein before complete equilibration of the indicator with the extravascular protein pool has been reached. The rate of protein equilibration depends on the amount of extravascular protein in the individual organs, as well as on the velocity of blood flow and, in all likelihood, on capillary permeability to protein.

Complete equilibration of labelled albumin with the total plasma albumin pool takes 2 to 5 days in humans, a little less in dogs [9, 12, 19, 25], and is considerably shorter in rats [17]. In the dog, equilibration of labelled protein between blood plasma and lymph is practically complete by the end of 7 to 12 hrs [35]. This indicates, on the other hand, that by the end of this period mixing of the indicator with the extravascular protein pool is complete. Therefore, the 24-hr. limit set in the present study must be sufficient for complete equilibration, leaving some doubts about the skin and the muscles only, as suggested by the morphologic appearance of the capillaries in these structures [2] and by the delayed colloid equilibration in their lymph [13, 20.]

It is more difficult to exclude the possibility that in certain organs significant amounts of labelled protein leave the intravascular space in the first three minutes. As to the kidneys, mixing of labelled serum albumin with extravascular albumin was demonstrable at three to fifteen minutes [6, 7]. The morphologic appearance of the capillaries suggests that this may apply to the liver as well. The fact remains that the differences between the 3-minute and 24-hr. intraorganic albumin spaces of the kidney and liver revealed by our earlier experiments in dogs [30, 31] as well as by the present study in rats were of unquestionable significance. The whole question dwindles, however, to a purely speculative issue by the fact that none of the organs showed any increase in the 24-hr. albumin space. The 3-minute, i.e. intravascular albumin space revealed no significant change, at least no definite reduction. It is therefore obvious that there cannot be any appreciable rise in the extravascular plasma protein volume calculated from the difference of the 3-min. and 24-hr. values either. It was on the other hand striking to find that the liver of irradiated animals was practically depleted of extravascular protein, a finding to be explained partly by an increase of the intravascular albumin space, partly by a reduction in total albumin. Though owing to the great scatter within the individual groups, the changes were not significant, they are none the less remarkable in view of the liver being the very site of albumin production. A minor increase in intravascular albumin space was detectable in most of the organs; this increase was significant in the kidney and spleen ($p < 0.01$), a finding interpreted as being due to dilatation of the small vessels or eventually to congestion.

The failure of demonstrating any increase in plasma protein in the individual organs definitely pleads against any significant change of capillary permeability. On the other hand, an excess of fluid was demonstrable in most of the tissues on the grounds of the reduced dry weight and protein nitrogen values. As a matter of fact, this might be a sign of oedema due to increased capillary permeability. Another possible cause of excessive water uptake may be a cellular destruction by the ionizing radiation. If, however, the water ex-

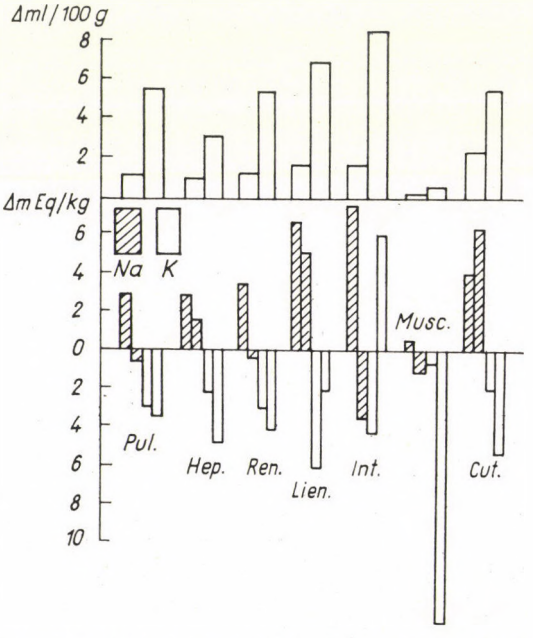

Fig. 4. Correlation of the expected and actual changes of fluid and electrolyte contents in irradiated animals. *Upper row:* Fluid content. Columns 1: Increase in fluid content of tissues. Columns 2: Computed tissular water uptake (ml/100 g wet weight). *Lower row:* Na and K concentration. Columns 1: Expected changes in concentration computed from tissular water uptake. Columns 2: Actual changes in concentration

cess is extracellular, in other words, produced by accumulation of oedema fluid, it must have the same electrolyte composition as the blood plasma.

Knowing the water uptake of the individual tissues it is easy to compute, with minor corrections according to the DONNAN equilibrium, to what degree the Na and K concentrations of that particular tissue are bound to change if the fluid excess is extracellular and if no significant cellular release of K and uptake of Na has taken place. The results of these calculations are shown in Fig. 4. The upper row represents the difference in the water content of tissues in the controls and irradiated animals, and the water uptake by the tissues of the irradiated animals computed from these values [ml/100 g]. The lower row shows the changes in the tissular concentrations of Na and K in the irradi-

ated animals. Here the first columns represent the calculated changes, expressing how the tissular electrolyte concentrations should have changed, were the fluid taken up by the tissues of the same electrolyte composition as the blood plasma. The second columns represent the actual values. It is seen that, with the exception of the spleen and of the skin, the actual concentrations reveal no distinct tendency to coincide with the expected values. The changes in the skin are, however, of doubtful informative value since this tissue is constituted for the greater part of interstitial fibres and not of cellular mass, and, in contrast to other tissues, its Na-concentration is higher than its K-concentration. At any rate, no correlations have been found between the expected and the actual changes in the Na-concentrations of the individual tissues ($r = 0.155$; $S_r = \pm 0.162$).

These findings suggest that the water excess found in the tissues is due to cellular, i.e. tissular breakdown rather than to an extracellular accumulation of fluid. This interpretation is consistent with our finding of reduced potassium concentrations in the individual tissues with the only exception of the intestinal wall, the reduction being significant in the muscles and the skin, at the borderline of significance in the liver and kidney, and not significant in the lung and spleen. In agreement with numerous observations [3, 4, 15] no change was demonstrable in the plasma concentrations of Na and K.

In sum, the present studies of water and electrolyte metabolism have also failed to confirm the extracellular origin, in other words the oedematous character, of water excess associated with sublethal total body irradiation, nor have they lent support to the claim that in radiation injury of this severity capillary permeability was increased to any significant degree. The results were consistent with the well-known fact that massive total body irradiation causes massive renal Na- and K-loss and K-depletion of the tissues sensitive to radiation [16].

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EFFECT OF LATERAL HYPOTHALAMIC LESION ON MATERNAL BEHAVIOUR AND FOETAL VITALITY IN THE RAT

By

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The influence of electrolytic lesion of the lateral (tuberal dorsolateral and infundibular ventrolateral) hypothalamic regions on maternal nest-building, retrieving, and nursing activities on one hand and on foetal vitality on the other has been studied and an attempt has been made to clarify the role of maternal behaviour and of foetal vitality by cross-fostering experiments. The studies included observations of maternal food and water intake, of body weight, and body temperature.

The mothers with hypothalamic lesion failed to develop even the fundamental patterns of maternal behaviour and the characteristic behavioural cycle after parturition. Even healthy offspring of healthy control mothers failed to initiate the pattern of maternal behaviour in these mothers. At the same time also their offspring was of poor vitality, unable to elicit nursing activity in control foster-mothers.

The important role of certain structures of the lateral hypothalamic region in the integration of maternal behaviour has been suggested by the authors.

In earlier studies we have shown that electrolytic injury of the hypothalamic region in rats during the third trimestre of gestation, while leaving the duration of gestation and parturition unaffected, leads to increased perinatal mortality, exceeding even 90 per cent on the second day after delivery if the tuberal dorsolateral and infundibular ventrolateral areas have been lesioned [1, 2].

It is still undecided which of the biologic mechanism, i.e. maternal behaviour, foetal vitality, or lactation, is affected by lateral hypothalamic lesions to a degree of leading to increased neonatal lethality. Since the lactation is organized primarily by anteromedial hypothalamic structures [3], we have confined these studies only to the aspects of maternal behaviour and foetal vitality. A study of these factors seemed also desirable on the grounds of our earlier observation that in pregnant animals with lateral hypothalamic lesion food and water intake was markedly reduced, a finding which may well connect the perinatal mortality with foetal maldevelopment. Further, these mothers did not build nests and left their young scattered about in the cage. Also the possibility might be raised that foetal vitality may have been too strongly affected to provide the necessary stimuli for the development and maintenance of maternal behaviour; an issue we have intended to clarify in cross-fostering experiments.

Material and methods

A total of 63 primiparous Wistar albino rats was used. The results of our earlier studies on 36 further litters have also been included [2].

Identification of the first day of gestation, observation of the whole course of delivery and electrolytic lesion to the respective hypothalamic areas between the 16th and 18th days of pregnancy were carried out as described previously [1, 2]. Food and water intake, rectal temperature and body weight of the test animals were recorded daily, as well as the average body weight of the young of the same litter.

Maternal behaviour was studied by a close observation of the nest-building, retrieving and nursing activities. The pregnant animals were confined to single cages whose posterior third was divided by a horizontal rimmed shelf into an upper and a lower compartment. Nest-building from soft paper-scraps was checked daily and intact nests were stirred up. The retrieving activity was studied by observing whether the mother was collecting the young into a nest immediately after delivery, furthermore, the young after weighing were placed once daily into the upper compartment of the cage and one hour later it was ascertained whether their mother had carried them back. The most characteristic sign of nursing behaviour was the nursing posture, when the mother was sprawling over the litter during the first post-partum days.

The following six groups were set up:
intact mothers (21)
with their own litter (Group I),
fostered litter from other intact mothers (Group II),
sham-operated mothers (35)
with their own litter (Group III),
fostered litter from mothers with lateral hypothalamic lesion (Group IV),
mothers after lateral hypothalamic lesion (27)
with their own litter (Group V),
fostered litter from sham-operated mothers (Group VI).

In the cross-fostered groups each mother had 4 or 5 fostered youngs delivered on identical days. Mortality rate was registered daily in each group.

Litters of sham-operated and lesioned mothers were compared in order to elucidate possible differences in the foetal vitality. For this purpose, 30 live-born young of 8 mothers with lateral hypothalamic lesion and 57 of 8 with sham-operation were kept in nests without their mother and the spontaneous daily mortality rate was recorded.

Results

Entirely normal maternal behaviour was found in Groups I, II and III (Table I). This means that from the soft paper-scraps provided for them they built nests into which they collected the young immediately after delivery, having freed them from the foetal membranes and the placenta. They invariably fetched and gathered them in the nests. Only one intact mother behaved in a different manner in that she built the nest in the upper compartment where the young had been placed and returned for every feeding. Nursing activity was unaffected in the intact and sham-operated animals, as reflected by foetal mortality (Fig. 1a and b). Perinatal death was relatively low in the first three days and there was no significant difference in this respect between the two groups.

The behaviour of mothers with lateral hypothalamic lesion was grossly deficient whether the young belonged to them (Table I, V) or to control sham-operated animals (Table I, VI). They built no nests and gnawed not only the

placenta and the envelops but also some of the young. They did not collect the young into nests but left them scattered about on the floor of the cage and instead of avoiding them, they trod them down. When the young were placed into the upper compartment they did not fetch them and showed no interest in them when they had been returned to the lower compartment. In brief, the lesioned mothers had lost their nursing activity, and 80 per cent of the live-born young died in the first two days of life (Fig. 1e). In this group no more than three mothers had still some of their young at the end of two days, and in these mothers the lesion of lateral hypothalamic regions in question was par-

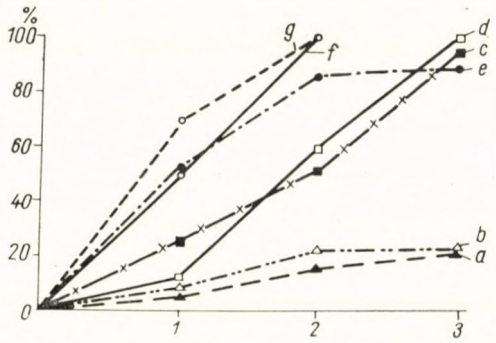

Fig. 1. Mortality of live-born young in the individual groups during the first 1 to 3 days after birth. Ordinate: Correlation of mortality with the number of live-births in per cent. Abscissa: Days. *a)* Intact mothers with young from other intact mothers. *b)* Sham-operated mothers with their own young. *c)* Mothers with lateral hypothalamic lesion with young born from control mothers. *d)* Young from control mothers isolated from mother, left without nursing. *e)* Mothers with lateral hypothalamic lesion with their own offspring. *f)* Young from mothers with lateral hypothalamic lesion, isolated from their mothers, left without nursing. *g)* Offspring of mothers with lateral hypothalamic lesion given to healthy foster mothers

Table I

Maternal behaviour in groups with lateral hypothalamic lesion and in control groups with own and fostered litter

No.	Groups		Nest-building	Retrieving of young from		Nursing
	Mothers	Young		upper compartment	floor	
I	intact	own	+	+	+	+
II	intact	intact foster	+	+	+	+
III	sham-operated	own	+	+	+	+
IV	sham-operated	injured foster	+	+	—	—
V	injured	own	—	—	—	—
VI	injured	sham-operated foster	—	—	—	—

tial or unilateral. It had no consequence whatsoever whether the young were from normal mothers, as by the end of the third day 96 per cent of the fostered young were dead and on the fourth day the only survivor, too, died (Fig. 1c).

The sham-operated animals showed a peculiar behaviour towards the offspring of mothers with lateral hypothalamic lesion (Table I, IV). They built nests in the manner observed in Groups I—III and retrieved the young from the upper compartment, but instead of collecting them into nests they left them scattered about and hurt them often by gnawing. They showed no nursing activity and these young died at the same rate (Fig. 1g) as those which had remained with their injured mothers (Fig. 1e; $p = 8$ per cent). By the end of the second day every such newborn animal was dead.

Mean birth weight of the live-born young was 5.63 g in the intact group, 5.45 g in the sham-operated group, and 4.92 g in the group with lateral hypothalamic lesion.

Spontaneous mortality is shown by graphs d and f of Fig. 1. It can be seen that young from mothers with lateral hypothalamic lesion (f) died at the same rate whether deprived from their mothers or remaining with them (e) or given to healthy foster-mothers (g). On the first day there was no significant difference between the three groups (f—g: $p = 12$ per cent; f—e: $p = 85$ per cent; g—e: $p = 8$ per cent); on the second day, total mortality in group e was 14 per cent less than in the other two groups, where mortality attained 100 per cent (f—e: $p = 4$ per cent; g—e: $p = 2$ per cent). Survival of the offspring of sham-operated mothers was longer (Fig. 1d) than of the offspring of lesioned mothers (f). They died at the same rate as the young of sham-operated animals given to lesioned mothers (c). The p-value computed on the basis of the difference between the two groups was 6 per cent on the first, 4 per cent on the second, and 14 per cent on the third day. Mean weight of one day old animals was 5.36 g in group d, and 4.96 g in group f; the difference was significant statistically (1 per cent $> p > 0.1$ per cent).

Maternal food and water intake, rectal temperature and body weight showed the same changes as those observed in our earlier experiments [2].

Discussion

Electrolytic lesion of the lateral (infundibular ventrolateral and tubular dorsolateral) hypothalamic regions in rats between the 16th and 18th days of gestation has been found to result in a disturbance of maternal behaviour marked by the loss of nest-building, retrieving and nursing activities. Consequently, in addition to the high mortality rate in the first six hours after delivery, the live-born young died during the first days of their lives. Apart from

the profound behavioural defect of the mothers, the reduced foetal vitality must also have played a part in the perinatal mortality. This was suggested not only by the low birth weight of the young born from mothers with hypothalamic lesion but also by the finding that the offspring of mothers with hypothalamic lesion died earlier when deprived of their mothers than did the control newborn animals under the same conditions.

It is well known that the post-delivery maternal behavioural pattern fails to develop if the young are isolated from the mother immediately after delivery and even if any of the behavioural features should have appeared, they rapidly subsided in the absence of foetal stimuli [11]. In the present studies we were looking for evidence which might possibly link up the postpartum behavioural defect in hypothalamus-damaged mothers with the poor vitality of the young which by this fact would fail to provide the necessary stimuli for the development and maintenance of maternal behaviour. We have found, indeed, in favour of this supposition, that healthy mothers when having their young exchanged for the offspring of mothers with lateral hypothalamic lesion, though being in possession of the basic characters of maternal behaviour, i.e. nest-building and retrieving activities, failed to develop, on the other hand, nursing behaviour toward these young which consequently died at the same rate as if they had been totally deprived of their mothers. The mortality rate of newborn rats kept together with their hypothalamus-damaged mothers was the same as in the two foregoing groups, but here even the basic characters of maternal behaviour remained entirely absent even toward fully viable offspring of normal mothers. Since there is evidence to suggest that survival of newborn animals with foster-mothers greatly depends on the time elapsed since delivery, in other words on the duration of lactation [4], exchange of the newborn animals was carried out in every instance immediately after cleansing from placenta and envelops, in the first 24 hours after delivery.

We thus believe to have lesioned such hypothalamic structures which are fundamental in the organization of maternal behaviour in the rat. There is no ground to connect behavioural disturbances induced in this manner with lactation disturbances, not only because there was no difference in the microscopic appearance of the mammary glands between the controls and the animals with hypothalamic lesion (unpublished data), but also because it has been shown that in the regulation of the hormones responsible for the release and maintenance of lactation the median structures of the hypothalamus are primarily involved [3, 7]. KURCZ [7] has shown that injury to the area of the ventromedial nucleus in the rat affects the mechanism of lactation while leaving the pattern of maternal instincts intact.

STAMM [12] found that extirpation of the median cortical (cingulate and retrosplenial) areas in the rat profoundly affects maternal behaviour without influencing lactation. In view of the abundance of hypothalamo-cortical

interconnections [9] there might exist functional links between the regions studied by STAMM [12] and those studied by us, moreover, that these two regions represent two different integrative levels of the same complex behavioural mechanism. It has to be mentioned that the regions studied by us largely cover those areas which have been found to be engaged, in the manner of a feeding centre, in the organization of feeding behaviour [9]. There is good reason to connect this finding with the poor vitality of the young born from mothers with lateral hypothalamic damage, since food and water intake is greatly reduced after lateral hypothalamic lesion, a finding which in itself may well account for the lower birth-weight and the poor vitality of the offspring, and leads us to believe that there is a reduction in the fluid content of the foetal tissues which, obviously, makes the young particularly vulnerable to perinatal dehydration. Though poor foetal vitality is thus an important factor of the excessive stillborn rate, primary disturbance of maternal behaviour is none the less significant. The fundamental characters of maternal behaviour known to exist in normal animals at the moment of delivery, regardless of external stimuli from the newborn [11], are adversely affected by the lateral hypothalamic lesion, to the extent that the mothers failed to make attempts at nest-building and, while gnawing off the placenta, they would gnaw their young as well.

It emerges from our experiments that the lateral hypothalamic regions play a large part in the integration of maternal behaviour, lesion to these areas being inhibitive to the development of the primary characters of maternal behaviour even in the presence of the necessary foetal stimuli. Apart from nervous structures, endocrine influences are also involved in shaping the pattern of maternal behaviour [5, 8, 10], obviously by definite interrelationships between these two regulatory systems. We have too little understanding of this intricate neuroendocrine mechanism to recognize its role at all phases of maternal behaviour pattern.

We are indebted to Mrs. J. Kovács for skilful assistance.

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STEROID TREATMENT OF LUPUS NEPHROPATHY

RESULTS OF 60 CASES

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The value of corticosteroids in SLE-nephropathy has been studied. Immediate response of the process to the drug was by no means convincing; this applies to the results of long-term treatment as well, even when viewed from the distance of six years. However, massive doses applied over long periods seemed to be more effective in halting the lethal progress of the process than sporadic courses with small doses, though the number of symptom-free cases was essentially the same in both groups and did not account for more than one third of the cases. Allowance must be made for possible spontaneous fluctuations of the process which may become manifest during prolonged periods of observation. The conclusion has been drawn that early massive-dose corticosteroid treatment in SLE holds the promise of a 50 per cent reduction in the morbidity and mortality rate of lupus nephritis.

The nephropathy of systemic lupus erythematosus (SLE) — which presents itself in the form of glomerulonephritis, nephrosis or as a mixture of both — is more resistant to treatment than any other manifestation of the disease. Though the application of corticosteroids has greatly improved the prognosis of SLE by the favourable response it generally elicits even in severe cases within a few days, it little affects the course of renal disease even when continued over long periods. In fact, renal disease has remained the prevalent cause of death in SLE. While in the view of some authors [7, 9] corticosteroids applied in adequately massive doses prevent the development of renal disease in SLE, other clinicians see no reason to share this optimism [4, 8, 10]. In our opinion, improvement of renal disease in SLE after corticoid therapy is too rare to be evaluable otherwise than by statistical analysis [5].

By the fact that the hazards of corticosteroid administration increase with its duration and with the doses, the advantages of long-term treatment with massive doses are made highly questionable. In October, 1965, the National Institute of Health has called upon the clinicians for co-operation by contributing evidence for a planned comprehensive evaluation of corticosteroid therapy in SLE-nephropathy [13].

The present paper reports upon our observations with corticoid treatment of renal disease associated with SLE.

Material and methods

In 60 out of 170 patients with SLE regularly followed up in the period 1952 to 1963, there were symptoms indicative of renal involvement. Cases with slight transitory renal episodes,

such as slight opalescence or a few erythrocytes in the urine, have not been included. Needle biopsy of the kidneys was adopted in 1958 and only in a few selected cases without attempting to check the results of corticosteroid treatment by this evidence. Our evaluation thus rests on the confrontation of the clinical findings of two periods. The first covers the six years between January, 1952, and December, 1957, the corticosteroid therapy was strictly confined to the actual periods of exacerbation and the lowest efficient dose was not exceeded. The second period includes another six years between January, 1958, and December, 1963, when by reason of various considerations including better facilities, every patient was immediately started on a massive-dose (termed today "immunosuppressive" course) with corticosteroids which was continued until the signs and symptoms were brought under control as far as possible. In case of residual signs the patients continued on the drug after discharge, and reported regularly at our special clinic. Thus, while in the first group corticosteroid treatment was restricted to the periods of exacerbation and even then the lowest efficient dose was prescribed, in the second group the exacerbations were treated with massive doses of 60 to 300 mg prednisolone daily, and in case of need treatment was continued after discharge with maintenance doses of 20 to 40 mg daily until freedom from symptoms was as complete as possible.

Twenty-eight out of the 60 patients with SLE-nephropathy belonged to the first, 32 to the second group.

Results

Table I shows that there is a definite difference between the results of sporadic treatment with low doses and of a systematic treatment with massive doses. Though renal death in Group II amounted to only 40 per cent of that in Group I, the cases where freedom from renal symptoms had been attained were hardly more in Group II than in Group I (10 : 8). In other words, by the reduction of mortality it was the number of slowly progressing cases which increased (18 in Group II *vs.* 10 in Group I) rather than that of the cures. The over-all figures in SLE show, however, that in Group I 28 cases out of 53, whereas in Group II only 32 out of 117 patients were or became nephropathic (Table II), in other words, in Group II renal disease was reduced to 50 per cent.

These figures strongly suggest that long-term treatment with massive corticosteroid doses inhibited the progression of nephropathy without actually

Table I

Outcome of SLE-nephropathy in 60 cases, in relation to the intensity of steroid treatment

SLE-nephropathy	Number of patients		
Outcome	I (1952—57) Sporadic low dosage	II (1958—63) Regular high dosage	Total
Death	10	4	14
Stagnation or slow progression	10	18	28
Freedom from symptoms	8	10	18
Total	28	32	60

Table II

Preventive value of corticosteroid treatment on nephropathy in SLE

Diagnosis	I (1952—57) Sporadic low dosage	II (1958—63) Regular high dosage	Total
Number of patients with SLE	53 (31.7%)	117 (68.3%)	170 (100%)
Incidence of nephropathy in these cases	28 (52.8%)	32 (27.2%)	60 (35%)

curing it. Accordingly, during the six year period of observation, the death rate of nephropathy decreased while the number of chronic cases increased.

Since from the year 1964 cytostatic (6-mercaptopurine) treatment has also been applied [6] in cases of severe nephropathy, we have no further basis for comparison.

Discussion

It may be inferred from the results that while corticosteroid treatment has no short-term effect on the nephropathy, prolonged application of massive doses perceptibly influences lethality when viewed over longer periods. The fact that as far as freedom from symptoms is concerned, a consistent schedule with massive doses has no significant advantage over sporadic treatment with minor doses, admits of two possible interpretations. 1) Either there are types which will respond to therapy at any rate, even to smaller doses if treatment is long enough; 2) or these are spontaneous remissions independent of treatment. The latter possibility is suggested by the complete spontaneous remissions witnessed in SLE, including cases of excessive severity, in the pre-corticosteroid era, though it is impossible today to give an estimate of the incidence of such spontaneous remissions, owing to the inadequacy of the standards on which the diagnosis of SLE was based prior to 1950.

It deserves, however, attention that 53 of our SLE patients were observed in the period 1952—57, and 117 in the period 1958—63, yet the number of cases with nephropathy was nearly identical in the two groups (28 and 32, respectively). Since the diagnostic criteria were the same in the two periods, in spite of the failure of corticoids to influence the nephropathy once it has developed, early application of massive corticoid doses might have some preventive value, as clearly suggested by the figures in Table II.

Our material, though fairly large, is still insufficient for the evaluation of corticoid treatment in respect to the individual types of SLE-nephropathy.

The present study raises certain issues in respect to nephrosis. Some types of this syndrome, particularly those associated with primary chronic nephritis, are regarded as "undifferentiated collagen" or as "monosystemic autoimmune"

disease, or, in general terms, as "primary immunopathologic process". What should be the therapeutic attitude in such cases? The obvious answer is that it must be the same as in SLE, since an immunopathologic process, whether generalized or monosystemic, calls necessarily for the same type of treatment and, as we expect, will also show the same pattern of response. In corticoid-resistant severe cases cytostatic agents (particularly 6-mercaptopurine) are given as the last resort [6, 12].

Evaluation of corticosteroid treatment in nephrosis must rest on firm diagnostic grounds. Though SLE may certainly appear as a monosystemic renal condition, there are on the other hand particular types of nephrosis, first of all lipid nephrosis in childhood, which show an excellent response to corticosteroids in 70 to 80 per cent of the cases [14]. Conversely, according to some authors [1, 3, 11] amyloid nephrosis precludes the use of corticosteroids.

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EFFECT OF HEXADIMETHRINE BROMIDE ON PITUITARY BLOOD FLOW IN RATS

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Injection of hexadimethrine bromide has been found to be followed by a reduction in blood pressure, cardiac output and adenohypophyseal blood flow in rats. The claim that the cause of pituitary necrosis induced by the compound lies in a reduction of regional blood flow, has been supported.

Post-partum necrosis of the anterior pituitary has been ascribed by SHEEHAN on the ground of microscopic examination of human material to an ischaemic mechanism (SHEEHAN [1937], SHEEHAN and STANFIELD [1961]). Intravenous administration of hexadimethrine bromide to rats has been found to induce changes of the same morphologic character as those seen in human pituitary necrosis (KOVÁCS et al. [1964, 1965 a 1966a, b]). A possible reduction of anterior pituitary blood flow by the substance in the laboratory animal would therefore support SHEEHAN's concept. It was this issue which we intended to clarify in the present study.

Method

Eighty female albino rats of identical strain kept on a standard diet were used. Twenty animals served as controls. The remaining 60 were treated intravenously with 5 mg hexadimethrine-bromide (Polybrene, Abbott), this dose having been found in earlier experiments to cause pituitary necrosis in about 50 per cent of the rats. Between the first and second, the second and fourth and the 12th and 24th hour after the injection the following parameters were determined: systolic blood pressure (mm Hg), cardiac output (ml/min/100 g body weight), weights of adeno- and neurohypophysis (mg/rat), adenohypophyseal and neurohypophyseal fraction of cardiac output, adenohypophyseal and neurohypophyseal blood flow (μl/min/mg adeno- and neurohypophysis), and vascular resistance of adeno- and neurohypophysis (10^3 cm · dyne · sec^{-5}/100 g adeno- and neurohypophysis).

The experiments were done according to SAPIRSTEIN (1958), GOLDMAN and SAPIRSTEIN (1958) and GOLDMAN (1963), as detailed in earlier studies (DÁVID et al. [1965], KOVÁCS et al. [1965b]).

After a 12-hour fast during which drinking water was allowed *ad libitum*, the rats were anaesthetized intraperitoneally by 45 mg/kg of phenobarbital, the femoral veins and the carotid artery of one side were exposed, and after an intravenous dose of 0.1 ml heparin, systolic blood pressure was measured directly with a mercury manometer connected to the carotid by a polyethylene catheter. This was followed by an injection of 0.2 ml ^{86}Rb Cl, corresponding to a total dose of 10 μC ^{86}Rb, into the femoral vein. Twenty to 60 sec. later 0.2 ml 1 per cent Evans-blue solution was injected into the femoral vein of the other side. After the injection of dye had been completed, arterial blood samples were collected at one second intervals, with the aid of a fraction collector through a cannula inserted into the carotid. When discoloration of the blood had ceased, the animals were killed by an intravenous dose of saturated KCl solu-

tion. The pituitary glands were removed immediately, the anterior lobe was severed from the neurohypophysis, both portions were weighed on an analytical balance to the accuracy of 0.1 mg. Then the adeno- and neurohypophyses were hydrolysed in 20 per cent KOH on a hot water bath, and their radioactivity was determined by means of thallium-activated hollow NaJ crystal. Cardiac output was determined by the dye-dilution technique of HAMILTON et al. (1932). The Evans-blue concentrations in arterial blood samples were measured with a Unicam-spectrophotometer at 620 mμ wave-length. The results were evaluated by Student's "t"-test.

Results

The results are shown in Table I. It may be seen that the blood pressure of the rats treated with hexadimethrine bromide was lower than that of the controls. The difference was the most distinct between the first and second hours and was still demonstrable between the 12th and 24th hrs. Cardiac output of the treated animals was lower than that of the controls; the difference was significant in the first few hours after the injection.

Adenohypophyseal weight slightly increased in the treated groups throughout, whereas the adenohypophyseal fraction of cardiac output showed hardly any change. A significant reduction in adenohypophyseal blood flow occurred in the first two hours and between the second and fourth hours, whereas 12 to 24 hrs after the injection the values were again in the range of those found in the controls. An increase in adenohypophyseal vascular resistance was found during the first few hours but the considerable scatter of the values did not permit to establish any significant deviation from the controls.

While in respect to weight, blood flow and vascular resistance, the neurohypophysis of the treated animals showed no significant deviation from the controls, the neurohypophyseal fraction of cardiac output was found to exceed significantly the control values at one to two, and two to four hours. It is, however, by no means certain whether this deviation has any biological significance. It deserves mention in this context that neurohypophyseal blood flow was many times that of the adenohypophyseal flow both in the test animals and in the controls. This is in agreement with the findings of GOLDMAN (1963) and with the results of our earlier studies (DÁVID et al. [1965]).

Discussion

It has been found that adenohypophyseal blood flow is reduced during the first hours after the administration of hexadimethrine bromide. This finding is obviously not applicable without due criticism to the pathogenesis of human pituitary necrosis, nevertheless it fits in remarkably well with the concept of SHEEHAN (SHEEHAN [1937], SHEEHAN and STANFIELD [1961]) linking up pituitary necrosis with a reduction in regional blood flow.

According to our earlier studies (KOVÁCS et al. [1964, 1966a]) intravenous administration of 5 mg hexadimethrine bromide causes in about 50 per cent of

the rats focal adenohypophyseal infarcts which take a few hours to be demonstrable microscopically. Allowance made for the individual variations, conclusive microscopic signs of necrosis are usually absent until the end of the first six hours. Large necrotic areas can also be seen with the naked eye whereas detection of minor infarcts requires microscopic examination.

In the present investigations no microscopic study was possible, as the pituitary glands had to be used for ^{86}Rb determination. On the basis of an earlier study of several hundred rat pituitaries after injection of hexadimethrine bromide, however, the compound must have had the usual effect in the present series, i.e. the induction of pituitary necrosis in about 50 per cent of the animals. This would seem to be discordant with the finding of a reduced adenohypophyseal blood flow in all but two animals contrasted with the controls during the first hours after the injection. The only possible explanation of this seeming discrepancy is that a reduction of regional blood flow does not necessarily lead to necrosis. In some cases, ischaemia may have been of shorter duration, while in others it was perhaps confined to smaller areas, therefore hypoxia was neither intense nor long enough to cause parenchymal destruction. It was, however, not possible to give experimental support to this explanation by the present studies, since SAPIRSTEIN's method does not measure blood flow continually; as the animal has to be sacrificed, the figures yielded by the method merely give an instantaneous picture of pituitary blood flow.

On the grounds of our earlier studies (KOVÁCS et al. [1964, 1965a, 1966a]), we should have found necrotic anterior pituitary areas in about 50 per cent of the animals 24 hours after the injection of hexadimethrine bromide. This seems to be at variance with our finding of normal blood flow at that phase, since, as generally known, infarcts are devoid of any blood flow. This we have been able to confirm by demonstrating that adenohypophyseal infarcts do not take up intracardially injected India ink (KOVÁCS et al., in press). These incongruent findings might be explained by the possibility that either the infarcts were too small or the method not sensitive enough to register minor changes. This again is contraindicated by our earlier rat experiments involving pituitary stalk lesion (DÁVID et al. [1965]). This operation has been found to induce pituitary infarcts involving about 50 per cent of the gland (DANIEL and PRICHARD [1956], LÁSZLÓ et al. [1962], ADAMS et al. [1963, 1964], GREEP [1963]). Reduction of pituitary blood flow was demonstrable in all of the animals affected in this manner. This would seem to suggest that vasodilatation, in other words, an acceleration of blood flow, ensues in the surviving tissues adjacent to the necrosed areas. Confirmation of this possibility requires microrheologic evidence *in vivo*.

The present results leave us with the problem of the unknown mechanism accounting for the transitory reduction in adenohypophyseal blood flow after the injection of hexadimethrine bromide. The fall of blood pressure and

Table I

Group	Number of animals	Body weight g	Blood pressure mm Hg	Cardiac output ml/min/100 g body weight	Adenohypophysis	
					Weight mg	Fraction of cardiac output per cent
I. Control	20	206 ±3.9*	122 ±2.0	31.8 ± 1.6	7.3 ±0.4	0.009 ±0.0005
II. Hexadimethrine bromide 1—2 hrs	20	219 ±5.9	77 ±5.1	24.4 ±0.9	8.7 ±0.6	0.007 ±0.0009
III. Hexadimethrine bromide 2—4 hrs	20	211 ±4.4	90 ±3.1	25.6 ±1.1	8,9 ±0.5	0.007 ±0.0004
IV. Hexadimethrine bromide 12—24 hrs	20	228 ±5.7	96 ±3.3	28.0 ±1.3	8.6 ±0.7	0.011 ±0.0016
Probability		I/II	$p \ll 0.001$	$p < 0.001$	$p > 0.05$	$p > 0.05$
		I/III	$p \ll 0.001$	$p < 0.01$	$0.02 > p > 0.01$	$p > 0.05$
		I/IV	$p < 0.001$	$p > 0.05$	$p > 0.05$	$p > 0.05$

* Standard error

cardiac output cannot be the only factors, first because in some animals blood flow was reduced though the said parameters were normal and second because if this supposition were true, severe hypoxaemic damage would have been found in other organs as well. In our view hexadimethrine bromide causes an arrest of pituitary circulation which results in hypoxaemic necrosis, though the present study has failed to throw light on the process directly responsible for this reaction. Ischaemia resulting in human anterior pituitary necrosis has been ascribed to embolism by SIMMONDS (1914), to thrombosis by REYE (1926) and SCHREINER (1955) and to vasospasm by SHEEHAN and STANFIELD (1961), whereas MCKAY et al. (1953), BEERNINK and MCKAY (1962) regard it as part of the Shwartzman-phenomenon. Necroses caused by hexadimethrine bromide leave the possibilities of vasospasm, stasis or thrombosis likewise open. Electron-microscopic studies (KOVÁCS et al. [1966b]) have failed to clarify this question, since the incriminated changes, i.e. vascular wall damage, stasis, thrombosis were already demonstrable in the pre-necrotic phase.

		Neurohypophysis			
Blood flow μl/min/mg adenohypophyseal weight	Vascular resistance 10^3 cm · dyne·$\cdot$sec^{-5}/100 g adenohypophyseal weight	Weight mg	Fraction of cardiac output per cent	Blood flow μl/min/mg/ neurohypophyseal weight	Vascular resistance 10^3cm·$\cdot$dyne· sec^{-5}/100 g neurohypophyseal weight
0.75 ±0.04	136.3 ±8.4	1.0 ±0.2	0.008 ±0.0008	6.00 ±0.61	18.7 ±2.2
0.40 ±0.05	167.8 ±15.9	1.1 ±0.1	0.014 ±0.0011	6.62 ±0.60	18.2 ±6.6
0.44 ±0.03	175.2 ±13.9	1.1 ±0.1	0.014 ±0.0012	7.28 ±0.66	14.6 ±3.7
0.81 ±0.11	114.6 ±11.9	1.2 ±0.1	0.009 ±0.0010	6.06 ±1.12	36.3 ±11.6
$p<0.001$	$p>0.05$	$p>0.05$	$p<0.001$	$p>0.05$	$p>0.05$
$p<0.001$	$0.05>p>0.02$	$p>0.05$	$p<0.001$	$p>0.05$	$p>0.05$
$p>0.05$	$p>0.05$	$p>0.05$	$p>0.05$	$p>0.05$	$p>0.05$

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ÜBER EINIGE AKTUELLE FRAGEN DER CHRONISCHEN PYELONEPHRITIS

Von

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Zwecks Vorbeugung der akuten Phase der Pyelonephritis empfiehlt es sich, die Katheterisierung zu vermeiden und Reihenuntersuchungen einzuführen. Die entsprechende Behandlung des akuten Prozesses bzw. die Kontrollierung der Patienten setzt die Möglichkeit der Entwicklung der chronischen Prozesse herab. Zwecks Behandlung und Kontrollierung der chronischen Kranken wird die Errichtung von Fürsorgeinstituten empfohlen. Im fortgeschrittenen Stadium des Prozesses kann mittels chronischer Hämodialyse das Leben der Patienten verlängert und auch verbessert werden.

In den vergangenen zwei Jahrzehnten stand die Pyelonephritis unverändert im Mittelpunkt des Interesses, was unter anderen den folgenden zuzuschreiben ist:

1. Die Häufigkeit der chronischen Pyelonephritis. Bei der Sektion findet sich in 4—15% der Fälle chronische Pyelonephritis [7, 8, 30, 34]. Hierbei sei erwähnt, daß die Pyelonephritis in der Mehrzahl der Fälle in vivo nicht erkannt wurde [7, 13, 22]. Auf die Bedeutung der latenten Pyelonephritis haben unter anderen ENDES und Mitarb. [6] sowie GÖMÖRI und SZENDEY [9] hingewiesen. Genannte Verfasser stellten anhand ihrer bioptischen und Sektionsbefunde fest, daß im Hintergrund der einfachen Hochdruckkrankheit, besonders wenn es sich um die maligne Form der Erkrankung handelt, nicht selten eine chronische Pyelonephritis steht. Bei der klinischen Untersuchung dieser Kranken erhob sich in den meisten Fällen nicht einmal der Verdacht einer chronischen Pyelonephritis.

2. Die Häufigkeit der Urämie. Laut der Statistik der Weltgesundheitsorganisation fallen jährlich 20—30 neue urämische Kranken auf 1 Million Einwohner, was in ungarischer Relation jährlich 200—300 neue Urämiefälle bedeutet.

3. Bakteriurie. Unter normalen Verhältnissen enthält der Harn keine oder durch Kontamination nur wenig Bakterien. Die Vermehrung der Bakterienzahl kann bereits auf Pyelonephritis deuten. Anhand der ausgedehnten Forschungsarbeit von KASS [14, 15, 16, 17] ist es heute bereits allgemein anerkannt, daß während eine Bakterienzahl unter 10 000/ml Harn Kontamination bedeutet, eine Bakterienzahl über 100 000/ml Harn (signifikante Bakteriurie) bereits auf Pyelonephritis hinweisen kann. Im Falle eines einmaligen positiven Resultats soll die Untersuchung wiederholt werden; falls sich die Signifikanz

abermals meldet (persistente Bakteriurie), empfiehlt es sich die Behandlung — selbst im Mangel anderer Symptome — zu beginnen, da es sich entweder bereits um Pyelonephritis handelt (latente Pyelonephritis) oder ein Prädisposition bedeutender Zustand vorliegt, der ohne Behandlung nicht selten zu Pyelonephritis führt [8, 15, 21, 29]. Die signifikante Bakteriurie stellt demnach ein klinisches Warnzeichen dar, das die Infektion der Harnwege noch vor dem Auftreten anderer Symptome ankündigt.

Angesichts der unter 1. und 2. angeführten düsteren Daten und der ermutigenden Angaben in Punkt 3. hielten wir die Erörterung einiger pathogenetischen Fragen der Pyelonephritis für angezeigt; das weitere Ziel vorliegender Arbeit war die Zusammenfassung jener therapeutischen Maßnahmen, die die Herabsetzung der Häufigkeit der akuten Pyelonephritis bezwecken bzw. dazu beitragen, daß die Progression der sich bereits entwickelten oder chronischen Prozesse zum Stillstand gebracht oder wenigstens verlangsamt werden kann.

Einige pathogenetische Beobachtungen

1. Bei unserer die menschlichen urologischen Erkrankungen reproduzierenden Versuchsserie wies das histologische Bild der Niere nach Infizierung der Blasenwand, des Hodens bzw. der Samenblase auf akute (Abb. 1) bzw. bei überlebenden Tieren auf chronische Pyelonephritis hin. Es wurde bewiesen, daß es sich nicht um eine kanalikulär, im Ureterlumen aszendierende, sondern um eine hämatogene Pyelonephritis handelt. Im Zustandekommen des Prozesses spielen die Erkrankung der regionalen Lymphknoten bzw. die darauffolgend entstandene Nierensaftkreislaufstörung eine Rolle [25]. Bei der Infizierung von 3 Bauchorganen (Darm, Gallenblase, Eileiter) erschien histologisch eine fleckige Nierenrindennekrose [26] (Abb. 2). Laut unserer unveröffentlichten tierexperimentellen Befunde sind nach Infizierung des Knochenmarks oder der Muskulatur schwere Nierenveränderungen (Tubulusepitheldegeneration, Zelldesquamation, stellenweise Nekrobiose) zu beobachten. Anhand unserer Experimente nahmen wir an, daß im Laufe der sich im Organismus abspielenden verschiedenen fiebrigen Infektionskrankheiten Nierenveränderungen zustandekommen können, die — falls sie nicht heilen — entweder in chronische Pyelonephritis übergehen oder — im Falle einer narbigen Heilung — dem Organismus eine Prädisposition bedeuten, was anläßlich späterer Infektionen die Entstehung einer erneuten Entzündung (Pyelonephritis) oder fleckiger Nekrose fördert. Die Ergebnisse weisen darauf hin, daß anläßlich der sich im Organismus abspielenden verschiedenen entzündlichen Prozesse die Möglichkeit einer sekundären hämatogenen Nierenveränderung auch zu erwägen ist. Diese Feststellung spricht für die Notwendigkeit der Harnuntersuchung: Auf Entzündung hinweisende Zeichen (Pyurie, Bakteriurie) oder lediglich signifikante Bakteriurie indizieren nebst der Behandlung der Grundkrankheit auch die

Behandlung bzw. Kontrollierung der Nierenveränderung. Es kann vorkommen, daß die zwecks Bekämpfung der Grundkrankheit angewandte Therapie (z. B. Antibiotika) auch den latenten, entzündlichen Prozeß der Niere teilweise oder vollkommen heilt.

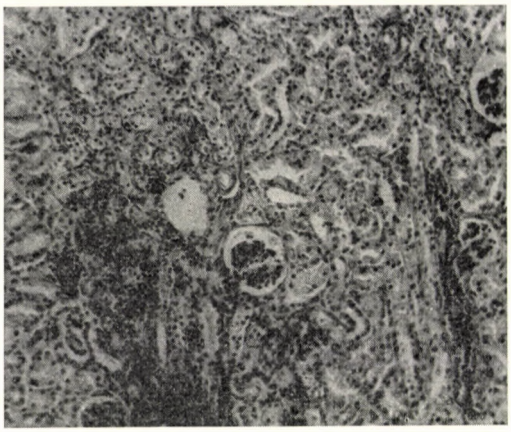

Abb. 1. Experimentelle akute Pyelonephritis beim Kaninchen: Exsudat in der Bowmanschen Kapsel, entzündliches Infiltrat im Interstitium

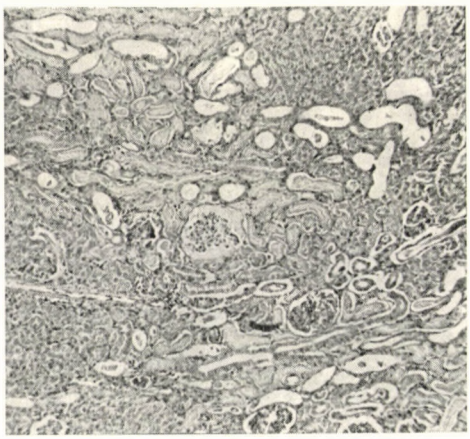

Abb. 2. Experimentelle fleckige Nierenrindennekrose beim Kaninchen

2. Pyelonephritis bzw. Bakteriurie im Kindesalter. Wie darauf zahlreiche Mitteilungen hinweisen, kommen bei Kindern Infektionen der Harnwege fast ebenso häufig vor wie die der Atemwege. Während Pyelonephritis nicht obstruktiven Ursprungs in erster Reihe die Altersgruppen zwischen 1—3 Jahren gefährdet, entwickelt sich die obstruktive Pyelonephritis zumeist bei 3—5jährigen Kindern [4, 19, 20]. In 1,5—4% der Fälle findet sich Bakteriurie. Häufig meldet sich pyelonephritische Aufflackerung und der Prozeß ist — ähnlich wie bei den Erwachsenen — oft symptomfrei. All dies spricht dafür, daß Bak-

teriurie oder Pyelonephritis auch im Kindesalter oft vorkommen, woraus folgt, daß ein Teil der bei den Erwachsenen auftretenden Pyelonephritiden bereits im Kindesalter begonnen hat.

3. Asymptomatische Bakteriurie in der Schwangerschaft. Laut der 1959 erschienenen Mitteilung von KASS [17] lag bei 6% der 4000 untersuchten Schwangeren signifikante Bakteriurie vor, während diese Prozentzahl bei 200 gesunden Unverheirateten lediglich 0,5% ausmachte. KASS nahm an, daß die Bakteriurie im zweiten Schwangerschaftsmonat begann. Bei Mehrgebärenden betrug die Prozentzahl der signifikanten Bakteriurie 11%. Bei 40% der nicht behandelten Schwangeren meldeten sich während der Schwangerschaft bzw. im Puerperium die klinischen Symptome der Pyelonephritis, in der behandelten Gruppe waren aber diese Zeichen in keinem Fall zu beobachten. Nach KASS kam es bei 24% der Schwangeren zu einer Frühgeburt, im Gegensatz zu der Kontrollgruppe, in der diese Prozentzahl nur 9% betrug. In dem 1500 Schwangere umfassenden Material von TURNER [32] meldete sich die signifikante Bakteriurie in 7% der Fälle, während dieses Symptom in der Kontrollgruppe nur bei 1% der Frauen vorzufinden war. Über ähnliche Befunde und pyelonephritische Folgen berichteten FORKMAN [8], SCHAMADEN [29], CUMMINS [5], PRÁT u. Mitarb. [23] usw.

Unsere mit der II. Frauenklinik der Medizinischen Universität Budapest gemeinsam durchgeführten unveröffentlichten Untersuchungen ergaben ähnliche Resultate. Mit Hilfe der zur Feststellung der signifikanten Bakteriurie dienenden Untersuchungen konnten wir in manchen Fällen keine Beschwerden verursachende Pyurie + Bakteriurie diagnostizieren.

Die angeführten Literaturangaben weisen darauf hin, daß infolge der in der Schwangerschaft häufig auftretenden signifikanten Bakteriurie sich im Laufe der Schwangerschaft oder im Puerperium eine manifeste Pyelonephritis entwickelt.

4. Bakteriurie und Pyelonephritis bei Diabetikern. Wie darauf zahlreiche Literaturangaben hinweisen, kommt die Pyelonephritis bei Zuckerkranken häufiger vor als bei nicht Diabetikern. Nach einigen Verfassern beträgt die Prozentzahl der an Pyelonephritis leidenden Zuckerkranken 18—20%. BALÁZS und Mitarb. [1] fanden eine 6%ige Häufigkeit; laut ROBBINS und TUCKER [28] ist die Todesursache bei 7,3% der Diabetiker Pyelonephritis, bei den nicht Diabetikern dagegen nur bei 1,3%. Im Verhältnis zu den übrigen Krankheiten beträgt die Häufigkeit der Pyelonephritis bei Diabetikern nach HETÉNYI [11] das Doppelte, nach BEESON [2] das Fünffache und nach KASS [16] das Vierfache, HANSEN [10] beobachtete anhand der Untersuchungen von 148 Diabetikern echte Bakteriurie bei 18,5% der zuckerkranken Frauen, und bei nur 3,7% der nicht Zuckerkranken; bei den Männern machten diese Prozentzahlen 7 bzw. 3% aus. Wie ersichtlich, kommt bei den Zuckerkranken häufig signifikante Bakteriurie bzw. Pyelonephritis vor.

5. Die Bedeutung der asymptomatischen Bakteriurie bei urologischen Erkrankungen. Nach unseren unveröffentlichten Untersuchungen ist der signifikanten Bakteriurie auch auf dem Gebiet der Urologie eine Bedeutung beizumessen (Nephroptose usw.).

6. Phenacetin und Pyelonephritis. Seit der 1953 erschienenen Mitteilung von SPÜHLER und ZOLLINGER [31] haben zahlreiche Forscher darauf hingewiesen, daß Phenacetinabusus häufig zu Pyelonephritis führt. Heute herrscht eher die Auffassung, daß das Pharmakon allein nicht Pyelonephritis verursacht sondern einen, das Anhaften der Bakterien in der Niere ermöglichenden Prädispositionszustand herbeiführt und auf diese Weise zur Entwicklung der Pyelonephritis beiträgt. In Ungarn scheint diese Frage von minderer Bedeutung zu sein.

7. Obstruktions-Pyelonephritis. Für diese bei Kindern häufig vorkommende Form der Pyelonephritis ist zumeist eine Entwicklungsanomalie der Niere bzw. des Harntransportsystems verantwortlich. Bei Erwachsenen bereitet die Diagnostizierung der entzündlichen Nierenprozesse im allgemeinen keine Schwierigkeit, da die auslösende Ursache (Stein, Gefäßanomalie, Prostatahypertrophie usw.) mit derartigen Beschwerden einhergeht, daß sich der Patient unbedingt zur fachärztlichen Untersuchung meldet.

8. Die Rolle der Katheterisierung bei der Infektion der Harnwege. Mit den Gefahren und Komplikationen der Katheterisierung befassen sich sämtliche Lehrbücher; in diesem Zusammenhang wollen wir aber auf die in der letzten Zeit stets lauter werdende Auffassung zahlreicher Verfasser hinweisen, daß schon nach einmaliger — zumeist nicht entsprechender — Katheterisierung die oberen Harnwege oft infiziert werden [3, 12 usw.]. Nach KASS [14] beträgt die Prozentzahl der nach Katheterisierung auftretenden Entzündung der oberen Harnwege 2—4%.

9. Vorangehende Nierenkrankheit als prädisponierender Faktor im Zustandekommen der Pyelonephritis. Zahlreiche Angaben sprechen dafür, daß zur Herbeiführung einer experimentellen Pyelonephritis die vorangehende Lädierung der Niere erforderlich ist [23, 25 usw.]. REUBI [24] hebt in der menschlichen Pathogenese die prädisponierende Rolle der chronischen Glomerulonephritis hervor, laut VARGA und TU-SÜJ-HAJ [33] ist in dieser Beziehung der Nephrose eine Bedeutung beizumessen.

Bei den anurischen Kranken unserer Kunstnierenstation waren außer Pyelonephritis folgende (der Pyelonephritis vorangehenden) Nierenkrankheiten zu verzeichnen: chronische Glomerulonephritis, Amyloidosis, Periarteriitis nodosa, Arteriosclerosis renis [27]. Über ähnliche Befunde berichtete auch FABRE [7]. Die pyelonephritischen Veränderungen der hypoplastischen Niere sind allbekannt.

10. Die Bedeutung der chronischen Pyelonephritis in der Entwicklung der akuten Oligo-Anurie. In unserem 492 Patienten umfassenden Material der

Kunstnierenstation war die auslösende Ursache der Oligo-Anurie in 122 Fällen (24,8%) die akute Aufflackerung eines chronischen Nierenprozesses. In 81 Fällen — was 67,1% der chronischen Nierenkranken bzw. 16,4% des gesamten Krankenmaterials ausmacht — war die Grundkrankheit chronische Pyelonephritis. Der Umstand, daß zahlreiche Kranken nichts über ihre vorangehende Nierenkrankheit wußten, spricht ebenfalls für die Bedeutung der latenten chronischen Pyelonephritis.

Werden diese willkürlich zusammengestellten pathogenetischen Faktoren und das Wesentliche des Prozesses zusammengefaßt, so ergibt sich, daß sich die Pyelonephritis in sämtlichen Lebensaltern — und so auch bei Kleinkindern — manifestieren kann, manchmal jedoch latent bleibt. In der Entwicklung der Krankheit können kongenitale und erworbene Nieren- bzw. Harntransportsystem-Veränderungen, verschiedene entzündliche Prozesse, Erkrankungen (Zuckerkrankheit), Schwangerschaft usw. eine Rolle spielen. Falls keine charakteristischen Symptome vorliegen, ist die Diagnose der akuten Phase häufig schwierig bzw. unmöglich. In Fällen, in denen die Pyelonephritis in der akuten Phase nicht definitiv heilt, sondern in die chronische Phase übergeht, kommt es früher oder später zur Zerstörung der Niere. Es kann vorkommen, daß die latente Pyelonephritis, die den Prozeß in Gang setzte, nur dann erkannt wird, wenn sich im Laufe der chronischen Phase als »erstes Symptom« Hochdruck, Urämie, Herzdekompensation bzw. Oligo-Anurie meldet.

Klinische Folgerungen

Unter Berücksichtigung der angeführten pathogenetischen Faktoren wollen wir die Wichtigkeit folgender prophylaktischer, diagnostischer und theoretischer Maßnahmen hervorheben:

1. Womögliche Vermeidung der Katheterisierung. In entsprechenden Fällen empfiehlt sich eher die Harnentnahme nach sorgfältigem Abspülen der Harnröhre (Mittelstrahlharn). In Fällen, in denen dieses Verfahren wegen irgendeiner Ursache nicht durchgeführt werden kann, müssen Kathetersterilisierung und Katheterisierung sorgfältigst vorgenommen werden.

2. Reihenuntersuchung. Wird die signifikante Bakteriurie im Kindesalter, während bzw. nach fiebrigen Erkrankungen, während der Schwangerschaft, bei Zuckerkranken, bei Entwicklungsanomalie bzw. Nephroptose usw. nachgewiesen, so kann der durch die Entwicklung einer eventuellen Pyelonephritis gefährdete Zustand der Niere rechtzeitig erkannt werden. Zur Klärung der übrigen Krankheiten bzw. Zustände, bei denen (oder wegen deren) signifikante Bakteriurie entstehen kann, sind weitere Forschungen erforderlich. Die Diagnostizierung der signifikanten Bakteriurie bedeutet gleichzeitig, daß anhand der in diesen Fällen unbedingt erforderlichen weiteren Nierenunter-

suchungen und Feststellung der Resistenz des Krankheitserregers (zumeist E. coli) die entsprechende Behandlung bzw. Kontrollierung des Patienten eingeleitet werden soll.

3. In manchen Fällen wird die diagnostizierte akute Pyelonephritis nicht in entsprechender Weise behandelt. Es kommt häufiger vor, daß falls nach der antibiotischen Kur die Beschwerden aufhören, die Patienten nicht weiter behandelt bzw. kontrolliert werden. In dieser Arbeit wollen wir auf die entsprechende Behandlung der akuten Pyelonephritis nicht eingehen, es sei jedoch betont, daß die akute Pyelonephritis eine lange Zeit hindurch behandelt und kontrolliert werden soll.

4. Die chronischen Prozesse beanspruchen eine sozusagen lebenslängliche Behandlung und Kontrollierung (Blutdruck, Anämie, Kreislauf, Salz- und Flüssigkeitshaushalt, Diät usw.). Diese Aufgabe sollten die in den Kliniken und Krankenhäusern errichteten Fürsorgeinstitute übernehmen.

5. Chronische Dialyse der arbeitsunfähigen urämischen Kranken.

Da die ausführliche Besprechung jedes einzelnen Punktes eine Mitteilung an und für sich ausfüllen würde, haben wir die mit der chronischen Pyelonephritis verbundenen Aufgaben nur schematisch zusammengefaßt. Das Ziel vorliegender Arbeit war, die Aufmerksamkeit auf den Umstand zu lenken, daß die Vorbeugung der chronischen Pyelonephritis, die Diagnostizierung und die entsprechende Behandlung der akuten und chronischen Phase für manche Kranken bessere Lebensverhältnisse bzw. die Verlängerung des Lebens bedeuten können.

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THE INFLUENCE OF OVARIECTOMY ON THE THYROID RESPONSE TO TSH IN THE MOUSE

By

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Ovariectomy was found to leave thyroid ^{131}I uptake and ^{131}I-tri-iodothyronine disappearance *in vivo* unaffected whereas it reduced the response to TSH of the thyroid. A transient reduction in pituitary TSH content was registered three weeks after ovariectomy, a finding interpreted as being due to an increased release of the hormone.

Recent studies concerning the effect of ovarian hormones on thyroid function [1, 2, 3] have clearly shown that the oestrogens enhance the capacity of blood plasma for binding thyroid hormones [4, 5]. In contrast, evidence as concerns the direct influence of ovarian hormones on the thyroid gland is not entirely consistent [1, 6, 7, 8], though this very issue is of an obvious practical significance, as illustrated by the prevalence of hyperthyroidism or hypothyroidism in females, particularly during the critical periods of ovarian activity.

Evidence is scanty in respect to the influence of ovarian activity on the thyroid response to thyrotrophic hormone (TSH) [1, 8, 9]. This has prompted us to study the influence of ovariectomy on thyroid activity, the thyroid response to TSH, and on pituitary TSH contents, in the mouse.

Material and methods

Female albino mice, approximately 20 g in weight, were kept on a iodine-poor diet. For the determination of thyroid ^{131}I uptake, 2 μCi ^{131}I was given intraperitoneally, the animals were killed 24 hours later, and the thyroid glands were removed and homogenized in 2 ml 2 N NaOH. Radioactivity was measured in a well-type scintillation-crystal and thyroid ^{131}I uptake in 24 hours was expressed in per cents of the injected activity.

For estimation of the disappearance rate of ^{131}I-tri-iodothyronine *in vivo*, the animals received 1 μCi/0.1 μg ^{131}I-tri-iodothyronine (Amersham) intravenously. Total activity in the animals was measured by means of a geometry-independent detector designed by KRASZNAI et al. [20] for the study of total body radioactivity in small animals. Values found at 1, 10, 24, and 48 hours, respectively, were expressed in per cents of the activity measured 10 minutes after administration of the compound.

Thyroid response to TSH was examined 3 and 12 weeks after ovariectomy by correlating the findings with those in mice subjected to a sham-operation. The effect of TSH on the thyroid was estimated on the basis of the method of MCKENZIE [10] adapted by us for this purpose [11]. If the test dose of TSH elicited a stimulatory response, then output of labelled hormone by the thyroid and consequently activity in the blood, increased. The standard TSH solution (lyophilized Ambinon-Organon) was prepared immediately before use. TSH was given intravenously in 0.5 ml fluid, in doses of 0.1, 0.2, 0.5, and 1.0 mU. Blood activity 2 hours after the intravenous injection of TSH was correlated with the mean 2-hour values (100 per cent) in mice injected with 0.5 ml of a 1 per cent saline solution of human serum albumin.

Finally, it was studied in 5 series whether ovariectomy had any effect on pituitary TSH content. The pituitaries of the animals were removed by the procedure of AMESBURY et al. [12], homogenized in saline and the TSH concentration was determined by the method of McKENZIE, using 300 albino mice. The pretreated test mice, grouped per 5 animals or more, were injected intravenously with 0.5 ml of an extract corresponding to 1/8 of the pituitary gland, whereas the control animals were given 1 per cent serum albumin in 0.5 ml saline, and the 2-hour activity values of the blood were compared. The reason for using 1/8 of the pituitary gland was that in our preliminary experiments we had found the thyroid stimulating effect of this amount sufficiently informative of pituitary TSH content.

Results

The upper section of Table I shows the 24-hour thyroid ^{131}I-uptake in per cent. It can be seen that ovariectomy did not affect these values. In the

Table I

Thyroid ^{131}I-uptake			
3 weeks after operation		12 weeks after operation	
Sham-operated	Ovariectomized	Sham-operated	Ovariectomized
22.8 ± 0.9 (12)	23.8 ± 0.9 (12)	24.0 ± 0.9 (10)	25.0 ± 0.7 (10)

24-hour total body activity expressed in per cents of the 10-minute value

3 weeks after operation	
Sham-operated	Ovariectomized
43.1 ± 1.2 (10)	40.9 ± 1.2 (10)

Upper section: Thyroid ^{131}I-uptake 24 hours after intraperitoneal injection of ^{131}I.
Lower section: ^{131}I-tri-iodo-thyronine disappearance rate *in vivo*. Reference value (100 per cent), whole-body activity 10 minutes after intravenous injection of the substance. In brackets, number of animals.

lower section the rate of ^{131}I-tri-iodothyronine disappearance is presented. Whole-body activity found 10 minutes after intravenous injection of the labelled hormone was taken as 100 per cent and the values obtained at 24 hours were expressed in per cent of this. It can be seen that — at least under the conditions of the present experiment — three weeks after ovariectomy the rate of disappearance *in vivo* of ^{131}I-tri-iodothyronine was unchanged. The graphs presented in Fig. 1 are consistent with this finding.

The figures seen in Table II reflect the effect of ovariectomy on the thyroid response to TSH. TSH given in doses of 0.1 and 0.2 mU elicited a slighter

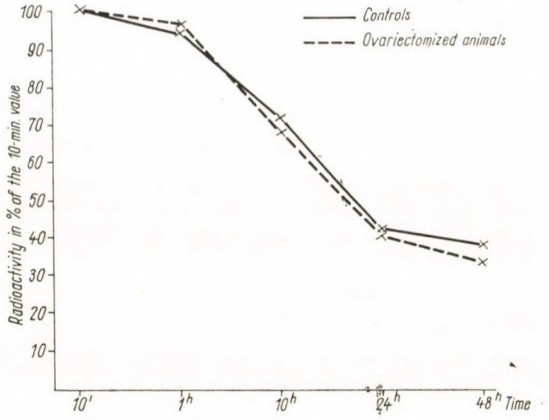

Fig. 1. ^{131}I-tri-iodothyronine disappearance rate *in vivo*. Mean values found in controls and in ovariectomized mice (10 animals per group). Reference value (100 per cent), whole-body activity 10 minutes after intravenous injection of the substance

Table II

Influence of ovariectomy on the thyroid response to TSH

Test dose	Blood activity expressed in per cents of the control (2-hr. value)	
TSH mU	3 weeks after operation	
	Sham-operated	Ovariectomized
0.1	220 ± 3 (14)	103 ± 3 (14) ***
0.1	252 ± 8 (15)	106 ± 2 (16) ***
0.1	260 ± 8 (16)	128 ± 4 (16) ***
0.1	213 ± 7 (10)	112 ± 6 (10) ***
0.2	322 ± 8 (17)	167 ± 3 (18) ***
0.2	275 ± 2 (17)	140 ± 3 (17) ***
0.5	520 ± 11 (16)	422 ± 4 (17) ***
1.0	670 ± 15 (16)	658 ± 9 (16)
TSH mU	12 weeks after operation	
	Sham-operated	Ovariectomized
0.1	253 ± 5 (15)	131 ± 3 (15)***

The control animals received 1 per cent human serum albumin in 0.5 ml saline. In brackets, the number of animals. In the cases marked with *** the difference between ovariectomized and sham-operated animals was highly significant ($p<0.1$).

stimulation on the response in ovariectomized than in sham-operated mice. The values found in the test animals and in the controls tended, however, to converge on increasing the dose, and after the administration of 1.0 mU of TSH the difference was no longer significant.

Table III presents the figures reflecting the influence of ovariectomy on pituitary TSH contents. In five series of experiments, the pituitary TSH contents were examined in five mice three weeks, in further five mice 12 weeks, after ovariectomy, each time against control groups of five sham-operated animals. The results were evaluated on the basis of two different standards. 1. The 2-hour activity found in the blood of test mice injected with 1/8 of the

Table III

Blood 2-hr activity in per cents of the control value

Series	1 Pituitary extract of sham-operated animals	2 Pituitary extract obtained 3 weeks after ovariectomy	3 Correlation of figures of column 2 to those of column 1, in per cent
I	332 ± 10	238 ± 11	71.8 ± 3.4***
II	315 ± 20	157 ± 6	49.8 ± 2.6***
III	252 ± 8	191 ± 6	75.7 ± 1.3***
IV	376 ± 18	212 ± 11	56.2 ± 1.3***
V	308 ± 7	245 ± 6	79.6 ± 1.2***

	Pituitary extract of sham-operated animals	Pituitary extract obtained 12 weeks after ovariectomy	Correlation of figures of column 2 to those of column 1, in per cent
I	315 ± 19	289 ± 10	91.9 ± 4.5
II	252 ± 8	246 ± 7	97.4 ± 1.5
III	248 ± 5	254 ± 6	102.4 ± 1.7
IV	382 ± 8	354 ± 8	92.5 ± 1.4**
V	304 ± 6	318 ± 6	104.6 ± 1.5*

Activity in test mice injected with pituitary of sham-operated and ovariectomized animals, expressed in per cent of the values found in the controls (Columns 1, 2), these having been given 1 per cent human serum albumin in 0.5 ml physiological saline. Column 3 shows the correlation of blood activity in mice injected with pituitary extract of ovariectomized mice, against the respective figures obtained in mice which had been injected with pituitary extract of sham-operated mice.

* difference significant ($p<5$)
** difference highly significant ($p<1$)
*** difference very highly significant ($p<0.1$)

pituitary substrate of (a) ovariectomized and (b) sham-operated mice was correlated with the 2-hour ^{131}I-activity of control mice (which had been injected with 1 per cent saline solution of serum albumin). (Table III, columns 1, 2.) 2. Two-hour blood activity of mice injected with pituitary extracts of sham-operated animals was taken as 100 per cent and the corresponding values found at 2 hours after injection with pituitaries of ovariectomized mice was correlated with this figure. Three weeks after ovariectomy the pituitary TSH concentrations were definitely reduced, in contrast to the findings in sham-operated animals. The difference was highly significant throughout ($p < 0.1$). Twelve weeks later no significant difference or none at all was demonstrable.

Discussion

The chief cause of the contradictions in the data concerning the influence of ovariectomy on thyroid iodine uptake lies in the use of different animal species and in the determinations having been made at different times after ovariectomy [13, 14, 15]. In the present studies, no abnormality in thyroid ^{131}I-uptake or in the elimination of injected ^{131}I-tri-iodothyronine was demonstrable three weeks after ovariectomy which indicates that at this point of time thyroid function was normal.

Noach [9] found that oestrogens enhanced the thyroid response to TSH. This is at variance with the assertion of Plunkett et al. [8], as well as of Beckers and DeVisscher [1] that ovariectomy combined with oestradiol administration reduced the excessive thyroid response to TSH. These experiments have, however, the shortcoming that thyroid response was not checked in control animals furthermore, that the doses of TSH were excessively high (10 IU). In the present study we were concerned with the response of ovariectomized mice, and to clarify this issue we applied smaller doses, close to the physiological range.

It emerges from our findings that three weeks after ovariectomy, thyroid response to TSH was reduced in spite of the normal ^{131}I-uptake. While 0.1 mU of TSH elicited a significant thyro-stimulating response in healthy mice, it failed to do so in ovariectomized animals. As the doses were increased the differences in the response between the controls and the ovariectomized animals diminished. 1.0 mU of TSH probably represented a stimulus which elicits the maximal response in 100 per cent of the animals.

If the thyroid gland fails to respond adequately to TSH the only means of keeping up the euthyroid state is a compensatory increase in pituitary TSH output. The findings of Bakke and Lawrence [16] indicate that a reduction in thyroid activity is followed by an increased pituitary TSH output, as reflected by the reduced pituitary TSH content three or four weeks after thyroidectomy. At a later stage pituitary TSH content was again normal. It there-

fore seemed pertinent to study the pituitary TSH-contents three weeks after ovariectomy when the thyroid gland shows a normal ^{131}I-uptake but a reduced response to TSH.

In the literature we find opposed views as regards the influence of ovariectomy or dosage of ovarian hormone on pituitary TSH-contents. While according to certain authors, castration leaves unaffected the pituitary TSH-concentration [17, 18], others observed a decrease in TSH output [19]. AMESBURY [12] found that in thyreoidectomized animals oestrogen administration did not affect pituitary TSH-concentration whereas in control animals it caused a reduction.

In the present study we found a reduction in pituitary TSH contents three weeks after ovariectomy. At twelve weeks there was, however, no significant difference in this respect between ovariectomized and control animals. This is interpreted in the following manner. The fact that three weeks after ovariectomy the thyroid response to TSH was reduced and yet the animal remained euthyroid was necessarily due to an increased pituitary TSH release. In fact, release of the hormone exceeded its production at first, as reflected by the low pituitary TSH contents three weeks after ovariectomy. When in the course of a few weeks TSH production had caught up with its release, the equilibrium thus reached was reflected by the identical figures found in the ovariectomized animals and the controls.

Though the results of these animal experiments cannot be applied to human pathology without due reservation, yet an analogy suggests itself between the present results and the conditions prevailing in menopause. It may be assumed that unless the reduced thyroid response associated with critical age is equilibrated by an increased pituitary TSH output, hypothyroidism will inevitably ensue, as indeed it is prevalent in climacteric women.

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CLINICAL TRIAL OF A NEW TYPE PROMPTLY ACTING PSYCHOENERGETIC AGENT (PHENYL-ISOPROPYL-METHYLPROPINYL-HCl, "E-250")

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E-250 comprises the clinical and pharmacological properties of amphetamine and MAO inhibitors.

The compound is a more potent MAO inhibitor than nialamide, its inhibitory effect is "irreversible". Its psychostimulatory action differs from that of amphetamine but is displayed immediately. It does not significantly enhance motility and basal metabolism. The drug has a hypotensive effect.

The most important feature of E-250 is that its action is prompt and lasting. Consequently it suffices to administer the drug for a short period of time. E-250 relieves depressed state immediately or it has no effect, in which respect it resembles the effect of ECT and differs remarkably from other antidepressants which need at least one, but in most cases two weeks to display their full effect.

E-250 is not a euphorizing agent, has no direct influence on psychic condition and general wellbeing, but exerts its action indirectly by enhancing activity and producing surplus energy.

On the basis of clinical observations in patients, the E-250 syndrome is characterized by an eagerness to act, a transitory sensation of heat without enhanced motility or reduced critical spirit, a relief of tiredness, prolonged feeling of alertness causing disturbed sleep and anorexia. All this is in contrast to the MAO inhibitors without acute action, i.e. to amphetamine which enhances motility and production.

The drug greatly enhances the drive — this effect manifests itself in the form of a striking sensation. Those who did not experience such a subjective sensation did not respond to the drug later either. Thus the target symptom of the drug is the lack of initiative (stupor, inhibition, negativism, etc).

A large number of new phenyl-alkylamine derivatives have been developed by KNOLL with the aim to enhance selectively certain effects and to eliminate some others of amphetamine (increased motility, enhanced basal metabolism, increased blood pressure, anorexigenic action, slight monoamino-oxidase inhibition, mild psychoenergetic action, etc.) One of these compounds is phenyl-isopropyl-methylpropinyl-HCl, (E-250), a promptly acting antidepressant with a mainly psychoenergetic effect.

One large group of psychoenergetics consists of dibenzazepine derivates, compounds related to phenothiazines with imipramine as their representative member. A series of compounds (des-methyl-imipramine, norimipramine) has been produced from the original substance without any marked increase in the effect (HAASE et al.).

Another and even larger group is that of the MAO inhibitors. The first in this group is the antituberculotic agent isonicotinic acid hydrazide, INH.

Table I

Characteristic effects of some psychoenergetic and psychostimulant agents (KNOLL)

	MAOI action	NE* action	Enhanced motility	Psycho-stimulant	Acute reserpine	Psycho-energetic, chronic reserpine	Euphor-izing
Imipramine	∅	++++	∅	∅	∅	∅	∅
Nialamide	+++	∅	∅	∅	∅	++++	∅
Amphetamine	+	+	++++	++++	++++	∅	+
E-250	++++	+	+	+++	+++	++++	∅
Pargyline	+++	+	∅	∅	+	+	∅

* Norepinephrin

Psychoenergetic agents similar to INH are classified among the hydrazide derivates iproniazid, isocarboxazid, phenelzin sulphate, nialamide. Another group is that of the phenylalkylamine derivates; their representatives are tranylcypromine, pargyline and E-250. It is not yet clear whether their enzyme inhibitory effect is the main pharmacodynamic one, or only a side effect.

Discussion

Beyond the conclusions which can be drawn from Table I, clinical experience has also justified the search for an antidepressant with immediate action (like that of E-250), as more than once convulsion treatment is needed in cases of depression, for inducing an immediate resolution of the inhibited state. However, the psychotic component of the action of members of both the azepine and the hydrazide groups manifests itself at the best a week after treatment has been started, but mostly two weeks later (PARE).

On the other hand, it is also known from PARE's data that there are certain "imipramine depressions" and imipramine resistant cases in which favorable results may be expected from MAO inhibitors. However, today we know that their representative compound, nialamide has not stood the test of time once the placebo effect of the new drug has passed; in clinical trials RICKELS estimated the drug's effect by an unfallible method on a scoring scale and has shown that it did not surpass the placebo effect.

Pharmacological analysis has justified the clinical trials with E-250. The compound possesses both a strong acute central nervous system stimulant action and a prolonged thymoleptic, psychoenergizing effect. The first may be attributed to the action of the amphetamine type component, the second indicates the MAOI character of the molecule.

E-250 differs from pargyline by its $CH—CH_3$ groups, these, however, not only change the compound's pharmacological character, but lend a differ-

ent chemical nature to the molecule which adopts stereoisomeric characteristics. Thus E-250 is a MAO inhibitory amphetamine derivative which reduces blood pressure without enhancing basal metabolism. Pargyline has a very slight psychoenergetic action and no psychostimulant effect whatsoever. It is nevertheless a new type of drugs in the treatment of hypertension, and though no antidepressant, is has at least no depressing effect either in contrast to reserpine.

As demonstrated in animal experiments by KNOLL, E-250 has a very pronounced acute psychostimulant action. In this respect it nearly parallels amphetamine and is more potent than phenmetrazine. Pargyline has no such effect neither does it affect motility.

Motility of the experimental animals (which is greatly enhanced by amphetamine) is hardly influenced by E-250 either. Thus a compound may display a psychostimulant action without enhancing motility.

A further important difference between amphetamine and E-250 is the fact that experimental depression induced by minor quantities of reserpine cannot be counteracted with amphetamine, while E-250 reliably inhibits it. The ineffectiveness of amphetamine in depressive states is well known from clinical practice. Finally, while amphetamine enhances basal metabolism, E-250 has no such effect and as a hypotensive it is similar to pargyline.

Toxicity tests

Subacute toxicity tests were carried out on dogs for four weeks by the manufacturing firm (Chinoin, Budapest). The doses varied between 1.25 and 2.50 mg/kg body weight.

Apart from minor anorexia and loss of weight no clinical symptoms were observed. Regular laboratory tests on animals showed no changes with the exception of the Mallen test which was positive after the second to third week. Autopsy findings indicated from the second week onwards gradually increasing severe focal hydropic degeneration of the liver and the focal fatty infiltration of the renal parenchyma.

On the basis of the pharmacological data we expected a primary action of the drug on drive, a stimulation of the activation system and, at the same time, a chronic antidepressent effect.

Consequently, we administered the drug first of all to negativistic patients, independently of the diagnosis, thus to subjects displaying inhibition, lack of initiative, scanty emotions and impulses, target symptoms connected with disturbed drive.

E-250 was not applied to patients with predominant anxiety, confused awareness, psychomotor excitement, disintegration or manic manifestations.

During treatment the patients were subjected to routine laboratory tests twice a week; blood counts, urine analysis, liver function tests, blood pressure, pulse rate and body temperature were regularly checked. The drug was not applied in cases of hepatic and renal lesions, with the exception of two patients with a history of hepatitis.

Table II

Psychoenergizing action of E-250

		Acute				Two months after discontinuation of treatment				
		+++	++	+	∅	+++	++	+	∅	(?) not known
Depression	15 men 2 women	11	—	3	3	4	4	5	4	(5)
Neurotic depression	3 men 2 women	3	2	—	—	1	2	—	?	(2)
Obsessional psychosis	2 men ∅ women	—	1	—	1	—	—	—	2	
Schizophrenic lack of initiative	8 men 1 woman	3	2	1	3	2	1	—	4	(2)
Paranoid schizophrenia	2 men ∅ women	1	—	—	1	—	—	—	2	
Normal	4 men ∅ women	uniform progress of the E-250 syndrome								
Total	34 men 5 women	25	5	4	8	7	7	—	12	(9)

Table II shows that the drug had a definite acute effect mainly on depressive patients in whom it resolved inhibition and the secondary psychic disturbances with practically the same speed as ECT.

Case reports

Case No. 1. A. Sz. male (born 1899), pensioner, had been treated eight years ago for depression with ECT. Before admission he had been depressed for months in a dejected, weary and despirited mood, had lost weight and been convinced to die shortly, had avoided people and been "afraid of himself". In the neurotic and later in the closed-ward nialamide and imipramine were administered but the condition deteriorated. After the first dose of E-250 the hitherto immobile state underwent a sudden change, "as if something had lifted me from my illness, lifted me or taken me out of it. I feel so light as if I had been lifted from a grip, as if I were completely healthy. I do not feel anything, only that all I had felt during my illness is suddenly over". On 0.05 g E-250 once a day for 5 days as the sole treatment the patient became active, looked after his fellow patients, slept well, appetite improved. He was discharged on trial and at a follow-up control three month later his condition was satisfactory. Diagnosis: involutionary depression.

Case No. 2. F. R. male (born 1919). Retarded development presumably after encephalitis in childhood. After primary schools he had become a butcher, performed his work and married. He was always silent and modest. Five years ago he fell ill, "became dumb", refused speech and to eat, was sad, wept. After ECT he regained his activity, but remained forgetful; he then worked as a school janitor and was considered a reliable, conscientious man. One month before admission he had suddenly grown quiet, refused to work, stayed at home, lost weight. As the condition did not change on imipramine treatment, E-250 was prescribed. On the fifth day of treatment his attitude changed, he was more active, his facial expressions were more suggestive, his mood improved, he began and carried on conversations with fellow patients, was more

lively during rounds and later asked for work. After two weeks treatment with E-250 (the longest uninterrupted E-250 therapy) he was discharged in an improved condition. Control after three months: took up work, where he performs satisfactorily, his condition is good. Diagnosis: imbecillity, depression.

Case No. 3. E. Cs. male (born 1919), grows vine, bachelor, lives with his sister's family. Three years ago he went through a depressive phase, attempted suicide with high voltage electric-current and later by stabbing himself in the chest, after which he received ECT. The condition improved, but "not the same person left the hospital as the one he had been before his illness", he was quiet, low spirited, his personality was paler, he was interested in his duty only and though actually not avoiding people did not seek their company. Before admission he indulged in self-accusation, believed himself to be a tax-swindler (exaggerating the real importance of the facts), avoided people, did not work, lay on his bed expecting his arrest, did not eat. At admission he was inhibited, indulged in self-accusations, was in a depressed mood. After a short ECT his condition remained unchanged. Subsequently E-250 was prescribed. On the third day of treatment he became active for the first time, asked for psychotherapy and conversation. On the next day he was cheerful, made friends with fellow patients and then proposed some quite realistic plans. After five days E-250 treatment was discontinued and the patient discharged on trial. As this was successful he was sent home. Control after three months revealed an invariably good condition. Diagnosis: depression with paranoid symptoms.

The actual effect of the drug on stuporous schizophrenics with lack of initiative is less pronounced but still quite marked.

Case No. 4. K. Z. male (born 1937), metallurgical worker, lives in good family conditions. He had been hospitalized three years ago because of persecution mania and acute psychosis, on ECT he cleared up slowly, and was discharged in an improved condition. He took up work but displayed less interest and emotions, his personality was less colourful than before. Three months before the second admission he had became restless, tense and though he mentioned no delusions and performed his work satisfactorily, he was kept under observation by his relatives. On the week before admission he refused to work, lay at home, took no interest in anything but did not complain. At admission his affected but conventional behaviour, lack of interest, total indifference and mildly manneristic psychomotor character were evident. Apart from tiredness he had no other complaints. In the ward he showed no initiative, but complied with all demands. He preferred to lie quietly and to meditate. Under the effect of the first dose of E-250 he suddenly took in things during lunch, began to speek to his fellow patients. On the third day he reports on improvement, wants to have visitors. On the fourth day he asks for a leave. On the fifth day he is allowed to leave on trial which is successful. Treatment is discontinued. After a second trial leave he is discharged. At a follow-up after two months the patient was well, he took up work. Diagnosis: postprocessual schizophrenic reaction.

Case No. 5. J. B. male (born 1910), graduate engineer, with good working and family conditions. Had had a psychotic episode before the admission. He was admitted in a state characterized by delusions of strange influences and poisoning. ECT was given. After discharge he yet had again hallucinations, smelt odours on himself, felt strange tastes to which he attached no delusional explanations. He knew himself to be ill, stayed at home and reported ill at his work. At home his condition was characterized beside olphactory and gustatory hallucinations by introversion, passivity, and awareness of being ill; he refused treatment and showed a complete lack of initiative. Under the influence of E-250 he recalled that since his illness 10 years ago he had had smell and taste sensations but had been able to perform his work excellently. He had mentioned his strange symptoms to nobody as he had attached no importance to them. Before the second admission it was not so much these strange sensations but a dejectedness and tiredness which had made him ill. During treatment his activity gradually returned. On the fifth day treatment was discontinued and he was allowed to leave on trial. A week later he was discharged in an active state, with faded hallucinations. He failed to report for a control examination. Diagnosis: postprocessual schizophrenia.

Increased consideration was given to the possible side effects. Many phrenotropic drugs including the MAO inhibitors are toxic. The most toxic of them is iproniazid. They affect primarily the liver and the hemopoietic organs. Imipramine jaundice and agranulocytosis probably are of allergic origin. It is well known that during or after MAOI treatment imipramine, reserpine or

biogenous amines elicit a "chesee reaction". Allergy may occur on the administration of any of the thymoleptics. Psychically hypomania, maniac conditions, psychotic exacerbation may also be expected (WAGENSOMMER). So we were precautious with the administration respectively to the interactions.

Table III

The side-effects of E-250

Age of patients	average 30 years youngest 16 years oldest 72 years	
Duration of treatment:	average 5 to 6 days (19 cases) shortest 1 day (9 cases) longest 16 days (1 case)	
Dosage: 1 0.05 g tablet orally once a day in the morning (in 4 cases 0.10 g)		
Loss of weight:	1 case	anorexia after two weeks treatment
Gain in weight:	4 cases	relieved depression and anorexia
Constipation:	0	
Diarrhoea:	0	
Gastric complaints:	2 cases	on the discontinuation of treatment the gastric symptoms disappeared
Hypertension:	1 case	acute anxiety
Hypotension:	26 cases	blood pressure, especially systolic pressure reduced by 10—30 mm Hg
Bradycardia:	0	
Tachycardia:	23 cases	insignificant, temporary
Sensation of heat:		obligatory transient side-effect
Dryness of mouth:	0	
Headache:	2 cases	temporary, characterized by a feeling of tension
Orthostatic collapse:	2 cases	both on the third day in patients older than fifty years
Changes in routine laboratory tests:	0	
Sleep disturbance:		obligatory temporary side-effect

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РЕЗЮМЕ

УСИЛЕНИЕ НЕРВНО-МЫШЕЧНОЙ ВОЗБУДИМОСТИ ПРИ НЕСПЕЦИФИЧЕСКИХ ЗАБОЛЕВАНИЯХ ДЫХАТЕЛЬНЫХ ОРГАНОВ У ДЕТЕЙ

М. ЧЕРНОВСКИ, Л. БРАНИЦКИ и В. ЧИКОШ

У 196 детей в возрасте от 3 до 14 лет, страдавших аллергическими или воспалительными болезнями бронхиальной системы, а также другими заболеваниями дыхательных органов, авторы очень часто наблюдали признаки усиления нервно-мышечной возбудимости. Для выяснения патофизиологии этого явления были проведены определения общего содержания кальция и неорганического фосфора в сыворотке, а также электрокардиографические исследования.

Согласно полученным результатам, при патологических процессах, сопровождающихся бронхиальной астмой, наиболее поразительным является гиперфосфатемия, всегда обнаруживаемая в активной фазе болезни, которая при бронхиальной астме составляет 8,1 ± 0,3 мг%, а при астматическом бронхите — 7,74 ± 0,24 мг%. Помимо того в значительной части случаев (50% или 38%) наблюдаются следующие изменения ЭКГ: удлинение интервала Q—T, высокий, острый и узкий зубец T.

Причиной указанных изменений, предположительно, является уменьшение ионизированного, диффундируемого кальция, возникающее одновременно с гиперфосфатемией. Указанное изменение минерального обмена веществ может вызвать усиление действия нервных импульсов, оказанного на меньшие дыхательные пути, в результате чего в повышенной мере проявляется одна из предпосылок возникновения астматического приступа.

О ГЕМОДИНАМИКЕ ЭССЕНЦИАЛЬНОЙ ГИПЕРТОНИИ

Р. ЮХЕМС

Сообщаются результаты экспериментальных исследований на 20 гипертониках, на группе здоровых лиц и на больных нейроциркуляционной астенией, проводившихся методом разведения красителей. В группе гипертоников периферическое сопротивление оказалось повышенным, а в группе больных функциональными болезнями сердца оно было пониженным. Минутный объем сердца и систолический объем сердца были у больных эссенциальной гипертонией уменьшенными, но если принимать во внимание возрастные различия, то, за исключением случаев декомпенсации сердца, эти величины можно признать нормальными.

ГЕМОДИНАМИЧЕСКОЕ ЗНАЧЕНИЕ ВЕНОЗНОГО БРОНХОПУЛЬМОНАЛЬНОГО КРОВООБРАЩЕНИЯ

К. ЛОЖАДИ и Т. ЛОНЬАИ

Роль венозных бронхопульмональных анастомозов при нормальных гемодинамических условиях еще невыяснена. При гипертонии системы легочной вены (напр., в случае порока двустворчатого клапана сердца) значительная часть крови легочной артерии попадает через бронхиальные вены в непарную вену, или в правое предсердие без протекания через левую половину сердца. Весьма вероятно, что этот шунт до определенной степени уменьшает застой в венозной системе легких, но в то же время еще более повышает нагрузку правой половины сердца. При помощи катетеризации непарной вены можно выявить ускорение бронхопульмонального венозного кровообращения.

ДЕЙСТВИЕ ПЕРХЛОРАТА КАЛИЯ НА ЩИТОВИДНУЮ ЖЕЛЕЗУ ПЛОДА

(Эксперименты на кроликах)

Л. ЛАМПЕ, Л. МОДИШ и А. ГЕЛ

Авторы давали беременным кроликам по 100 мг перхлората калия на кг веса тела в день и, сравнивая с данными контрольных животных, исследовали на 21-й и 28-й день беременности: а) вес, б) гистологическую картину, в) гистохимические реакции и г) соотношение ацинусов, эпителия и стромы щитовидной железы матерей и плодов.

Согласно полученным результатам, перхлорат проникает через плацентарный барьер и вызывает в щитовидной железе плода те же гистологические изменения, что и в щитовидной железе матери. При действии перхлората вес фетальных щитовидных желез увеличивается сравнительно более, чем вес щитодиной железы матери.

НАРУШЕНИЕ УГЛЕВОДНОГО ОБМЕНА ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ ИНФАРКТОИДНОЙ КАРДИОПАТИИ

Ч. ХЕДЬВАРИ, Э. НЕМЕШАНСКИ и Й. ШОШ

Авторы исследовали на изолированных препаратах сердца углеводный обмен, после поражения сердца в результате кардиопатогенного режима питания. В группе животных с кардиопатией было установлено нормальное поголощение глюкозы, уменьшение выделения молочной кислоты и повышенное выделение пировиноградной кислоты. Изменения обмена веществ обуславливаются, по всей вероятности, понижением дегидрогеназной активности лактата, нарушением цикла Сент-Дьерди—Кребса и, в конечном итоге, уменьшением митохондриальной функции.

ИЗМЕРЕНИЕ ДАВЛЕНИЯ НА РАЗЛИЧНЫХ УЧАСТКАХ ЛИМФАТИЧЕСКОЙ СИСТЕМЫ

Д. САБО и Ж. МАДЬАР

Авторы проводили у 23 собак под хлоралозовым наркозом измерения среднего давления на отдельных участках лимфатической системы, а именно, на шее: в грудном протоке, в шейном стволе и в правом лимфатическом стволе; в грудной клетке: в отводящем лимфатическом стволе сердца и в бронхомедиастинальном стволе; в брюшной полости: в лимфатическом сосуде печени, в кишечном стволе, в люмбальном стволе, а также в лимфатических сосудах почек. Результаты измерений предоставили лишнее доказательство того, что центростремительный ток лимфы поддерживается временными факторами, оказывающими местное действие в отдельных областях, а не градиентами давления постоянного характера.

ДЕЙСТВИЕ ОБЛУЧЕНИЯ ВСЕГО ТЕЛА НА ПРОНИЦАЕМОСТЬ КАПИЛЛЯРОВ

Исследование содержания воды и электролитов в организме и внутриорганных пространств плазменных белков

Д. САБО, Е. ШАРМАИ и Ж. МАДЬЯР

У крысы после рентгеновского облучения всего тела дозой 650 р концентрация плазменных белков, а также сухой вес и содержание азота в отдельных органах понизились.

Концентрация натрия и калия в плазме крови не изменилась. Содержание натрия в тканях селезенки и кожи повысилось, в кишках оно уменьшилось, а содержание калия в общем уменьшилось.

Тканевое пространство плазменных белков («внутрисосудистое альбуминовое пространство»), при измерении, спустя 3 минуты после внутривенного введения альбумина, меченого J^{131} у облученных животных, как правило, увеличивалось, однако, достоверное изменение было получено лишь в почках и в селезенке. Спустя 24 часа после введения меченого альбумина, пространство J^{131} («общее пространство внутриорганного альбумина»)

не увеличилось ни в одном органе. Поразительно, что в печени внутрисосудистое пространство и общее пространство внутриорганного альбумина были одинаковыми, и что внесосудистый альбумин исчез. Анализ полученных данных показывает, что под влиянием облучения не происходит повышения количества внесосудистых плазменных белков, и повышение содержания воды в тканях не является результатом отека, повышения количества внеклеточной воды, так как между изменением содержания воды и содержания натрия не существует корреляции. Следовательно, результаты исследований авторов не подтверждают положение о повышении капиллярной проницаемости.

ИЗМЕНЕНИЯ ПОВЕДЕНИЯ МАТЕРЕЙ И ЖИЗНЕСПОСОБНОСТИ ПОТОМСТВА У КРЫС ПОСЛЕ ПОВРЕЖДЕНИЯ ЛАТЕРАЛЬНЫХ ОБЛАСТЕЙ ГИПОТАЛАМУСА

З. АВАР и Э. МОНОШ

Авторы исследовали действие электрического повреждения латеральных областей гипоталамуса (regio tuberalis dorsolateralis, regio infundibularis ventrolateralis) у беременных крыс на активность матерей по строению гнезда («retrieving»), по уходу за своими детенышами («nursing») и на жизнеспособность потомства. Было исследован также вопрос о том, каким образом изменяются поведение матерей и смертность детенышей в случае замены потомства матерей с поврежденным гипоталамусом на потомство контрольных животных. Помимо этого измерялись также потребление пищи и воды крысами и вес и температура их тела.

У матерей с поврежденным гипоталамусом не наблюдалось и элементов материнского поведения, не развилось послеродового характерного цикла материнского поведения, и детеныши родились с меньшей жизнеспособностью. Контрольные крысы не могли выращивать потомство матерей с повреждением гипоталамуса, и у крыс с повреждением гипоталамуса под влиянием детенышей контрольных матерей также не наблюдалось развития материнского поведения.

По мнению авторов, отдельные структуры латеральной части гипоталамуса играют большую роль в развитии материнского поведения.

ЭФФЕКТИВНОСТЬ СТЕРОИДНОЙ ТЕРАПИИ ПРИ ЛЮПОЗНОМ НЕФРИТЕ ИЛИ НЕФРОЗЕ

Д. ПЕТРАНЬИ и А. ЛЁВЕИ

Сообщаются данные к эффективности кортикостероидов при нефропатии с синдромом системной красной волчанки. Лечебное действие преднизолона неубедительное не только непосредственно после его применения, но оно ненадежное и по истечении 6 лет. Длительное лечение большими дозами преднизолона в отношении смертельного прогрессиврования болезни, повидимому, более благоприятно, чем нерегулярная дача небольших доз; однако, в двух группах число больных, ставших бессимптомными, не показывает существенных отклонений и составляет приблизительно лишь одну треть случаев. В этом результате, наблюдаемом в течение длительного лечения, повидимому, имеет значение также самопроизвольная флюктуация процесса.

Более важным является то установление автора, что соответствующее патронирование больных с синдромом системной красной волчанки и их энергичное лечение кортикостероидами выраженно (приблизительно на половину) снижает вероятность развития тяжелой люпозной нефропатии.

ДЕЙСТВИЕ БРОМИДА ГЕКСАДИМЕТРИНА НА ГИПОФИЗАРНОЕ КРОВООБРАЩЕНИЕ

К. КОВАЧ, Л. ЧЕРНАИ и Ш. БЕРТЕНЬИ

После введения бромида гексадиметрина у крысы понижаются кровяное давление, минутный объем крови и кровообращение в аденогипофизе. Эти данные подтверждают мнение авторов, согласно которому некроз аденогипофиза, наблюдаемый после дачи бромида гексадиметрина, обуславливается местным нарушением кровообращения.

НЕКОТОРЫЕ АКТУАЛЬНЫЕ ПРОБЛЕМЫ ХРОНИЧЕСКОГО ПИЕЛОНЕФРИТА

Ф. РЕНЬИ-ВАМОШ

Ввиду частоты заболеваемости хроническим пиелонефритом, этот патологический процесс находится в центре внимания исследователей. Для профилактики острой фазы следует проводить серийные исследования и обойтись без катетеризации. Возможности развития хронического процесса уменьшаются при соответствующем лечении острого процесса и контрольном обследовании больных. Для лечения и контрольного обследования больных с хроническим пиелонефритом автор рекомендует учреждение диспансеров. В далеко зашедшей стадии болезни продление жизни и облегчение состояния больных возможны лишь при помощи хронического гемодиализа.

ДЕЙСТВИЕ ЭКСТИРПАЦИИ ЯИЧНИКОВ У МЫШЕЙ НА ЧУВСТВИТЕЛЬНОСТЬ ЩИТОВИДНОЙ ЖЕЛЕЗЫ К ТИРЕОТРОПНОМУ ГОРМОНУ

Я. ФЁЛДЕШ, И. КРАСНАИ, Э. ГЕСТЕШИ и И. ТАКАЧ

После удаления яичников у мышей поглощение J^{131} щитовидной железой остается неизменным. Скорость исчезновения трийодтиронина, меченого J^{131} при внутривенном введении in vivo также не показывает изменения. В то же время наблюдается уменьшение чувствительности щитовидной железы к тиреотропному гормону. Спустя 3 недели после удаления яичников, содержание тиреотропного гормона в гипофизе мышей временно уменьшалось: это явление авторы приводят в связь с нарастанием выделения гормона.

ПРИМЕНЕНИЕ В ПСИХИАТРИИ НОВОГО ПСИХОЭНЕРГЕТИЧЕСКОГО ПРЕПАРАТА БЫСТРОГО ДЕЙСТВИЯ (ФЕНИЛИЗОПРОПИЛ-МЕТИЛПРОПИНИЛ-АМИН HCL «Е—250»)

Э. ВАРГА и Л. ТРИНГЕР

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REFRAKTIONSUNTERSUCHUNGEN

Von

L. MOLNÁR

OPHTHALMOLOGISCHE ABTEILUNG (CHEFARZT: DR. L. MOLNÁR) DES KOMITATSKRANKENHAUSES, DEBRECEN

(Eingegangen am 10. Mai 1966)

Bei 84 Frühgeborenen, 81 Neugeborenen, 426 Säuglingen, 48 Kindern unter 2 Jahren sowie der Gesamtbevölkerung der Gemeinde Újléta wurden anhand der in Zykloplegie bestimmten Lichtbrechung die Änderung der Refraktion in den verschiedenen Lebensaltern, die Gestaltung der Brechkraft der Kornea sowie die Verteilung der Myopie, Emmetropie und Hypermetropie untersucht. Auf Grund der ermittelten Angaben werden einige ophthalmologische Faktoren (durchschnittliche Refraktion bei der Geburt bzw. im Laufe der späteren Jahre, Entwicklung der Emmetropisation und des optischen Apparats) bekanntgegeben, ferner anhand von bei 111 schielenden Kindern durchgeführten Refraktionsuntersuchungen die Ätiologie der einzelnen Strabismustypen erörtert.

Die Refraktion ergibt sich aus dem Bulbusdurchmesser sowie der Lichtbrechung der Kornea und der Linse. IWANOW [2] fand anläßlich der bei 1monatigen Säuglingen und zweieinhalbmonatigen Kadavern durchgeführten Untersuchungen einen durchschnittlichen Bulbusdurchmesser von 18,5 mm. Die bei Leichenaugen vorgenommenen Messungen sind ziemlich ungenau, in vivo kann aber die Untersuchung nur bei eingeübten Personen und mit Hilfe spezialer Einrichtungen vorgenommen werden. Bei der RUSHTONschen Methode [8] wird die Längsachse dunkeladaptierter Augen mit Hilfe von Röntgenstrahlen bestimmt. Der Apparat von DASCHEWSKIJ [1] besteht aus einer Spaltlampe und einem Photoapparat. Von den verfertigten Filmstreifen können der Krümmungsradius der Kornea, die Tiefe der Vorderkammer, der Krümmungsradius der vorderen und hinteren Linsenoberfläche, die Dicke der Linse und die Refraktion des Auges abgelesen werden. Die Umrechnung der Lichtbrechung der einzelnen optischen Elemente in Dioptriewerte erfolgt mit Hilfe spezialer Tabellen; die Methode eignet sich auch zur Bestimmung der Linsenrefraktion. Da uns keine solche Instrumente zur Verfügung standen, unterließen wir die Untersuchung dieser beiden Faktoren und bestimmten lediglich die Lichtbrechung sowie die Brechkraft der Kornea. Anhand der bei Früh- und Neugeborenen ermittelten Angaben analysierten wir die Gestaltung der Brechkraft der Hornhaut in den ersten Lebensjahren und die Änderung der Refraktion von der Geburt bis zum Greisenalter.

Material und Methodik

Die Lichtbrechung von 84 Frühgeborenen, 81 Neugeborenen, 426 Säuglingen, 48 Kindern unter 2 Jahren sowie der Gesamtbevölkerung (2084 Personen) der Gemeinde Újléta wurde in Zykloplegie bestimmt; auf diese Weise konnten wir die Änderung der Refraktion in einer geschlossenen Population beobachten. Um feststellen zu können, inwiefern die von anderen Verfassern bestimmten Achsenlängswerte die Entwicklung der endgültigen Gesamtlichtbrechungskraft und der Refraktion unter Berücksichtigung der Lichtbrechung der Linse beeinflußten, bestimmten wir außerdem die Brechkraft der Kornea bei 114 Früh- und Neugeborenen bzw. Kindern unter zwei Jahren. Diese Untersuchungen ermöglichten die Feststellung der prozentualen Verteilung der Myopie, Emmetropie und Hypermetropie bzw. der pathologisch auffaßbaren Ametropie in den einzelnen Altersgruppen. Die ermittelten Werte wurden mit den Refraktionswerten von 111 schielenden 3—10jährigen Kindern verglichen, was die ätiologische Bewertung der einzelnen Strabismustypen ermöglichte.

Die zur Untersuchung der Lichtbrechung von Neugeborenen, Säuglingen und Kleinkindern dienende Methode wurde bereits bekanntgegeben [7]. Bei der Messung der Brechkraft der Hornhaut wurde das Auge nach vorangehender Tetrakain-Anästhesie mit der Hand oder mit dem DESMARRESschen Löffel geöffnet. In etwa 30% der Fälle erwies sich die Fixierung des Bulbus durch Festhaltung der Konjunktiva mit einer Pinzette (3 mm vom Limbus entfernt, in der Höhe von 3 und 9 Uhr) für erforderlich.

Ergebnisse und Diskussion

Der durchschnittliche Refraktionswert schwankt bei Neugeborenen nach den verschiedenen Verfassern zwischen + 1,8 und + 5,3 D. Die unterschiedlichen Ergebnisse sind wahrscheinlich auf die Verschiedenheit der Bestimmungsmethoden zurückzuführen. Wir fanden bei Neu- bzw. Frühgeborenen Werte von +3,2 bzw. +3,9 D; die durchschnittliche Refraktion dürfte somit bei Neugeborenen +3,0 D und bei Frühgeborenen +4,0 D ausmachen.

Die Brechkraft der Kornea beträgt nach den Messungen von SEEFELDER [9] 50,5 D, nach unseren Bestimmungen 48,4 D; bei den Frühgeborenen fanden wir einen Durchschnittswert von 53,1 D. Laut DASCHEWSKIJ [1] macht die gesamte Brechkraft des Auges bei der Geburt 81,0 D aus, woraus zu folgern ist, daß die Brechkraft der 18 mm langen Linse des Neugeborenen über eine Brechkraft von etwa 30,0—35,0 D verfügt, was aber selbstverständlich auch von der im Auge eingenommenen Position der Linse abhängt. Nach den Schätzungen von DASCHEWSKIJ [1] beträgt dieser Wert 35,0 D.

Unmittelbar nach der Geburt beginnt die Verminderung der Refraktion, die Emmetropisation. Die Frage, in welchem Lebensalter sich die Entwicklung des Bulbus abschließt, ist noch ungeklärt. Die endgültige Größe des Bulbus entwickelt sich nach STEIGER [10] anläßlich der Variation einiger, voneinander unabhängiger, vererblicher optischer Konstanten (Brechkraft der Kornea und Linse, Achsenlänge). Die Kombination dieser erblichen Konstanten werden selbstverständlich auch von peristatischen Einflüssen (geographische Lage, Strahlenwirkung, Klima, gesellschaftliche Einflüsse usw.) gefördert bzw. gehemmt. Die Frage, wann sich die Entwicklung des Bulbus abschließt, hat STEIGER [10] jedenfalls nicht beantwortet. KETTESY [3] meint, daß auch der

Zeitpunkt, in dem sich das Längenwachstum des Auges abschließt, vererblich ist. Seiner Ansicht nach gibt es Augen, bei denen die Stabilisation bis zum Ende des 2. Lebensjahrs eintritt, während in anderen Fällen das Längenwachstum nur Ende des ersten Dezenniums oder gleichzeitig mit der körperlichen Vollentwicklung zum Stillstand kommt.

Das Wachstum des Augapfels ist im ersten Lebensjahr am intensivsten, da sich die Fixation, die koordinierte Augen- und Handbewegungen etwa im 4.—6. Monat entwickeln, während die Akkomodation nach unseren Untersuchungen gleichzeitig mit dem PURKINJE-SANSONschen Spiegelbild, im 7.—9. Monat für vollentwickelt gilt. Mit fortschreitendem Alter vermindert sich die Gesamtbrechkraft des Auges: Dies würde die Steigerung der bereits bei der Geburt bestehenden Hypermetropie zur Folge haben, wenn gleichzeitig der Längsdurchmesser des Bulbus nicht in noch bedeutenderem Maße anwachsen würde. Da nach IWANOW [2] dieser Wert am Ende des Säuglingsalters 22 mm ausmacht, wurde die Längsachse im Laufe eines Jahres um 4 mm länger, was einem Dioptriewert von —9,0 D (1,0 D = 0,44 mm) entspricht. Wie auf Tab. 1

Tabelle 1

Durchschnittliche Refraktion und Brechkraft der Kornea bei Frühgeborenen, Neugeborenen, Säuglingen und Kindern unter zwei Jahren

Lebensalter	Zahl der Fälle	Durchschnittliche Refraktion	Zahl der Fälle	Durchschnittliche Brechkraft der Kornea
Frühgeborene: 2—7 Tage	84	+3,9 D	16	53,1 D
Neugeborene: 2—7 Tage	81	+3,2 D	8	48,4 D
Frühgeborene und Säuglinge: 8—30 Tage	148	+2,9 D	13	45,9 D
Säuglinge: 1—6 Monate	165	+2,7 D	27	45,7—46,1 D
Säuglinge: 6—12 Monate	113	+2,6 D	11	44,8 D
Kinder: 1—2 Jahre	48	+2,4 D	39	43,9—44,2 D

ersichtlich, wurde der Refraktionsdurchschnitt der Kornea um +0,6 D, die Brechkraft der Kornea bei Frühgeborenen um +8,3 D und bei Neugeborenen um +3,6 D niedriger. Demnach muß sich im ersten Lebensjahr vornehmlich die Brechkraft der Linse in ziemlich bedeutendem Maße vermindern, damit die Projektion auf der Retina punktartig werde.

Anhand unserer Untersuchungen ist die Brechkraft der Hornhaut am Ende des 2. Lebensjahrs annähernd endgültig. Der Längsdurchmesser des Auges beträgt nach IWANOW [2] 23 mm, während den Angaben von DASCHEW-

Tabelle 2

Verteilung der Hypermetropie, Emmetropie und Myopie bei Neugeborenen
(Literaturangaben)

Verfasser	Hypermetropie	Emmetropie	Myopie
	%		
Titow [12]	80,9	11,5	7,6
Selenskij und Klebanskaja [15]	94,4	4,0	1,6
Utkin [14]	97,9	1,75	0,35
Molnár [7]	92,2	4,8	3,0
Durchschnitt	91,35	5,51	3,14

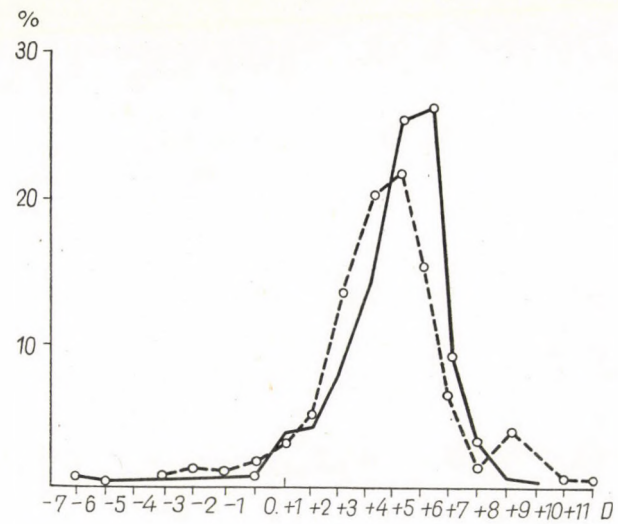

Abb. 1

——— Durchschnittlicher Dioptriewert: +3,95 (Selenskij und Mitarb.)
------- Durchschnittlicher Dioptriewert: +3,63 (Verfasser)

Refraktionszustand von Neugeborenen und

	—7	—6	—5	—4	—3	—2	—1	0
Zahl der Fälle[1]	3	1	—	—	—	—	4	20
%	0,6	0,2	—	—	—	—	0,8	4,0
Zahl der Fälle[2]	—	—	—	1	2	2	3	5
%	—	—	—	0,6	1,2	1,2	1,8	3,0

[1] Angaben von Selenskij und Mitarb.
[2] Angaben vom Verfasser

SKIJ [1] gemäß die Brechkraft der Linse 19,8 D und die Gesamtbrechkraft 59,9 D ausmachen; dies bedeutet, daß im 3. Lebensjahr die Entwicklung des optischen Apparats fast abgeschlossen ist. Abgesehen von der Brechungsametropie kann demnach nur davon die Rede sein, daß zwischen der Refraktion und der Änderung des Augenlängsdurchmessers ein Zusammenhang besteht.

Aus Tab. 2 geht hervor, daß 3,14% der Neugeborenen myop, 5,51% emmetrop und 91,35% hypermetrop sind. Anhand der Untersuchung von 2398 Neugeborenen gelangte STEIGER [10] zu folgenden Feststellungen: Myopie 0,4%, Emmetropie 0,9%, Hypermetropie 98,7%. In der Gruppe der Übersichtigen gestalteten sich die Dioptriewerte folgendermaßen: 1—2 D = 55,7%, 3—4 D = 31,4%, 5—6 D = 10,2%, 7—8 D = 1,4%.

Die Ergebnisse der von uns und von SELENSKIJ sowie KLEBANSKAJA [15] bei Neugeborenen durchgeführten Lichtbrechungsuntersuchungen veranschaulicht Abb. 1. Wie ersichtlich, kamen Myopie in 4,8%, Emmetropie in 3,0% der Fälle vor. Dioptriewerte zwischen +0,5 D und +2,5 D waren in 19,4% der Fälle, zwischen +3,0 D und 5,5 D in 59% der Fälle und über +6,0 D in 13,8% der Fälle zu verzeichnen. Die Verteilung ist möglicherweise etwas gekünstelt, die sich in den späteren Jahren vollziehende Veränderung der Refraktion kann aber nur auf diese Weise registriert werden. Ansonsten stimmt diese Aufteilung mit der Hypermetropie-Gruppierung (geringgradig = fakultativ, mittelgradig = relativ, hochgradig = absolut) sozusagen überein.

Die Verschiebung in Richtung der Emmetropie ist in Tab. 3 und 4 dargestellt. Die Angaben der Tab. 3 zeigen, daß sich am Ende des 1. Lebensjahrs die Prozentzahl der hochgradigen Hypermetropie fast um 50%, die der Fälle mit mittelgradiger Hypermetropie lediglich um 2—4% vermindert, während die Anzahl der Fälle mit geringgradiger Hypermetropie zunimmt und die der myopischen Fälle unverändert bleibt. Tab. 4, in der die Angaben der Gesamtbevölkerung der Gemeinde Újléta angegeben sind, veranschaulicht die Werte der älteren Jahrgänge. Obwohl zwischen den Daten der beiden Tabellen gewisse Abweichungen vorliegen, kann festgestellt werden, daß die Ametropie in 2—3%

Frühgeborenen am ersten Lebenstag

+1	+2	+3	+4	+5	+6	+7	+8	+9	+10	+11
21	41	73	130	138	48	16	3	2	—	—
4.2	8,2	14,6	26,0	27,6	9,6	3,2	0,6	0,4	—	—
9	23	34	37	26	11	3	7	—	1	1
5,5	13,9	20,7	22,5	15,8	6,6	1,8	4,2	—	0,6	0,6

Tabelle 3

Veränderung der Hypermetropie, Emmetropie und Myopie in den verschiedenen Altern

Lebensalter	Zahl der Fälle	M	E	Zwischen +0,5 D und +2,5 D	Zwischen +3,0 D und +5,5 D	Über +6,0 D
		%				
Neugeborene: 2—7 Tage	165	4,8	3,0	19,4	59,0	13,8
Säuglinge: 7—30 Tage	148	4,0	5,4	26,3	54,2	10,1
Säuglinge: 2—12 Monate	278	4,6	5,7	27,0	57,4	5,3
Kinder: 1—2 Jahre	48	4,2	18,7	22,6	52,4	2,1
3—5jährige Kinder	83	6,0	17,0	36,1	38,5	2,4
schielende Kinder	60	—	—	20,0	64,4	15,6
6—10jährige Kinder	45	7,9	21,0	37,4	31,4	2,3
schielende Kinder	51	—	—	31,3	54,2	14,5
Kinder: 11—15 Jahre	63	8,4	39,5	29,0	21,2	1,9
Alter in Jahren 16—30	128	8,7	51,9	21,0	16,2	2,2

M = Myopie
E = Emmetropie

der hochgradigen Hypermetropie-Fälle am Ende des 3. Lebensjahrs zum Stillstand kommt und auch in den späteren Jahren unverändert bleibt. Gleichzeitig vermindert sich auch die Anzahl der Fälle mit mittelgradiger Hypermetropie fast auf die Hälfte; von diesem Zeitpunkt ab bis zum 15.—20. Lebensjahr kann jährlich eine durchschnittliche Verminderung von 2—3% beobachtet werden, bei der Altersgruppe 15—20 ist schließlich ein konstanter Wert — mittelgradige Hypermetropie in 15% der Fälle — zu verzeichnen. Es erhebt sich die Frage, ob sich die Hypermetropie in diesen Fällen bis zum Ende der Pubertät stufenweise entwickelte oder — analog zur hochgradigen Hypermetropie — bereits Ende des 3. Lebensjahrs zustandekam. Um dies entscheiden zu können, haben wir die Refraktion von 60, 3jährigen und 51, 7jährigen schielenden Kindern gemessen; in den folgenden 4 Jahren wurde die Gestaltung der Lichtbrechung dieser Patienten regelmäßig kontrolliert (Tab. 5). Es ergab sich, daß im Verlauf von 4 Jahren die Hypermetropie bei 59,4% der zur ersten Gruppe gehörenden Fälle (im Material von MIYASHITA [6] betrug diese Prozentzahl 54,5%) und in 63,3% der Patienten der zweiten Gruppe entweder stationär blieb oder sich steigerte. Dementsprechend ist die Anzahl der Fälle, in denen sich eine Änderung der Hypermetropie vollzog, bei schielenden und nicht-

Tabelle 4

Prozentuale und dem Lebensalter entsprechende Verteilung der gering-, mittel- und hochgradigen Hypermetropie, Emmetrepie und Myopie bei 1848 Kindern und Erwachsenen
(Bevölkerung der Gemeinde Újléta)

Lebensalter (Jahre)	M	E	Zwischen +0,5 D und +2,5 D	Zwischen +3,0 D und +5,5 D	Über +6,0 D
	%				
1	3,4	—	27,5	58,8	10,3
2	—	—	26,0	69,7	4,3
3	4,5	—	45,4	45,6	4,5
4	6,3	—	59,4	31,2	3,1
5	5,3	4,3	56,4	29,0	5,0
6	4,9	4,9	60,7	25,7	3,8
7	3,6	5,0	61,7	26,2	3,5
8	4,4	5,2	63,4	23,9	3,1
9	4,7	5,6	64,7	22,0	3,0
10	4,5	18,0	52,2	22,3	3,0
11	3,9	12,5	59,1	21,0	3,5
12	4,1	9,6	63,5	20,9	1,9
13	7,5	14,2	58,4	16,6	3,3
14	7,2	17,7	53,3	19,0	2,8
15—20	4,6	48,1	29,7	15,0	2,6
21—39	4,6	52,9	26,5	13,6	2,4
40—59	4,8	38,9	43,3	10,9	2,1

M = Myopie
E = Emmetropie

schielenden Kindern annähernd identisch, woraus folgt, daß obwohl die Verminderung der relativen Hypermetropie nach dem 3. Lebensjahr am bedeutendsten ist, es keineswegs behauptet werden kann, daß sich in den erwähnten Fällen die mittelgradige Hypermetropie bereits im Alter von 3 Jahren entwickelte. Dies könnte nur mit einer von der Geburt bis zur Pubertät dauernden, fortlaufenden Untersuchung der Betroffenen entschieden werden. Wird jedoch

die Hypermetropie-Verteilung der 3—6jährigen und 6—10jährigen schielenden Kinder mit den Angaben der nicht-schielenden Kinder ähnlichen Alters verglichen (Tab. 3), so ergibt sich, daß im Verhältnis zu den nicht-schielenden, in beiden Gruppen der Schielenden die Anzahl der hochgradigen Hypermetropie-Fälle 7mal, die der mittelgradigen 2mal höher lag, während die geringgradige Hypermetropie seltener vorkam.

Tabelle 5

Veränderung der Übersichtigkeit von Schielenden während 4 Jahren

	Gruppe I 3—6jährige Kinder (60 Fälle) %	Gruppe II 7—10jährige Kinder (51 Fälle) %
Unveränderte Hypermetropie	47,7	59,4
Steigerung der Hypermetropie	11,7	3,9
Verminderung der Hypermetropie	35,7	21,0
Emmetropisation der Hypermetropie	3,3	9,8
Myopisation der Hypermetropie	1,6	5,9

Anhand dieser Feststellung dürfte angenommen werden, daß sich die relative Hypermetropie, analog zur absoluten Hypermetropie, sehr früh, bereits im 2.—3. Lebensjahr stabilisiert und auf diese Weise im Zustandekommen des Strabismus accomodativus eine kausale Rolle spielt.

Die in Abb. 2 angeführten Angaben bezüglich der Gestaltung der Myopie in Újléta weisen darauf hin, daß unter den 4jährigen Kindern 2/3 der Kurzsichtigen an gering- bzw. mittelgradiger Myopie und 1/3 an maligner Myopie leiden. Bei den 10—14jährigen ist die Anzahl der an der letzterwähnten Veränderung leidenden sozusagen unverändert, während die Prozentzahl der ersterwähnten Fälle ständig zunimmt und sich fast verdoppelt. Etwa zur Zeit der Pubertät stabilisiert sich ein Teil der geringgradigen Myopien, in einer Anzahl der Fälle (etwa 0,5%) entwickelt sich dagegen, wahrscheinlich wegen Brechungsprobleme, eine Emmetropie. Der Umstand, daß sich im 18.—20. Lebensjahr die Mehrzahl der mittelgradigen Myopie-Fälle stabilisiert, bei 0,5—0,8% der Kinder es jedoch zur bösartigen Entartung kommt, erklärt auch die Tatsache, daß die Kurve der gering- bzw. mittelgradigen Kurzsichtigkeit nach dem 10.—14. Lebensjahr eine 1,3%ige Verminderung zeigt. MIYASHITA [6], der die Refraktion von 59 Medizin-Studentinnen 4 Jahre hindurch kontrollierte, stellte fest, daß die Kurzsichtigkeit in 62% der Fälle stationär und in 38% der Fälle progressiv war (nach unseren Ergebnissen betrugen diese Prozentzahlen 67 bzw. 33%). In Újléta machten die gering- und mittelgradige Myopie bei Personen ähnlichen Alters 62% un die maligne 38% aus. In den spä-

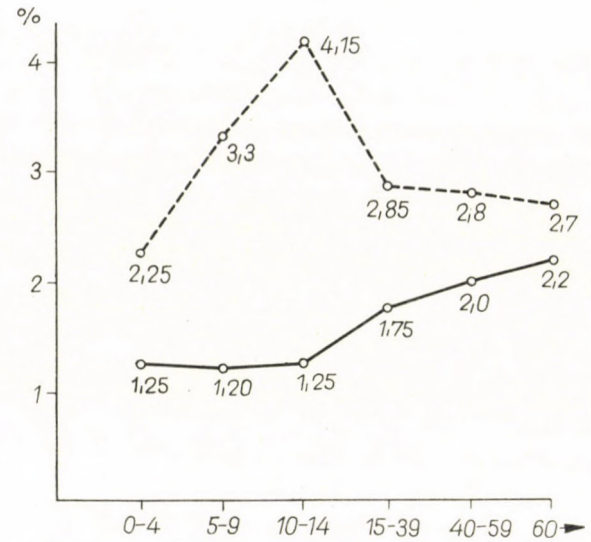

Abb. 2. Prozentuales Vorkommen der Myopie in den verschiedenen Lebensaltern (Újléta, 2084 Einwohner)
– – – – Kleingradige und mittelgradige Myopie; ——— maligne Myopie

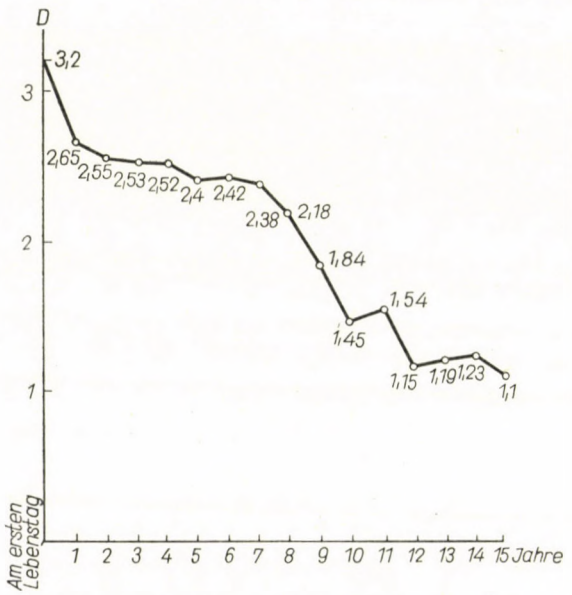

Abb. 3. Durchschnittliche Refraktionswerte der Neugeborenen, Säuglinge und Kinder (Újléta)

teren Jahren wies die letzterwähnte Veränderung eine ansteigende und die ersterwähnte eine abnehmende Tendenz auf, woraus folgt, daß sich die Kurzsichtigkeit in einer minimalen Prozentzahl der Fälle selbst nach dem 18.—24.

Lebensjahr nicht stabilisiert. Natürlich besteht bei diesen Kurzsichtigen auch eine Fundusveränderung.

Nach den Literaturangaben nimmt die Zahl der Kurzsichtigen stets zu. UTKIN [14] fand diese Veränderung bei 15,58% der 16jährigen Kindern vor (geringgradige Myopie 72,37% dieser Fälle, mittelgradige, d. h. Dioptriewerte

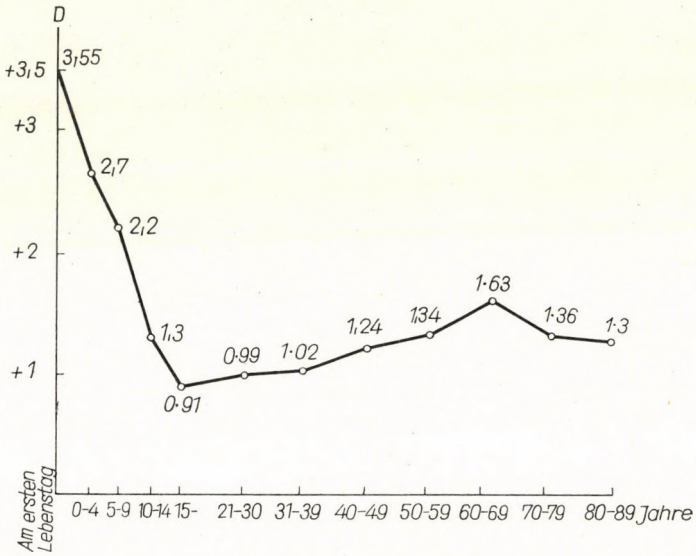

Abb. 4. Verteilung der durchschnittlichen Refraktion in den verschiedenen Lebensaltern (Újléta, Gesamtbevölkerung)

zwischen —3,0 und —6,0 D, in 22,37% und maligne Myopie in 5,26% der Fälle). Seiner Ansicht nach progrediert die Kurzsichtigkeit mittleren Grades vom 9. Lebensjahr an und geht im Alter von 13 Jahren bereits in eine hochgradige Form über. Anläßlich der statistischen Bewertung von 1156 Kurzsichtigen stellte UENO [13] fest, daß es sich in der Mehrzahl der vor dem 10. Lebensjahr auftretenden Myopie-Fälle um eine genetisch determinierte angeborene Anomalie handelt. In 20,3% der Fälle konnte die Vererbung nachgewiesen werden. Bei den Frauen kam die Kurzsichtigkeit häufiger vor als bei den Männern.

Laut zahlreicher Verfasser ist eine Lichtbrechung von +5,0 D noch für normal zu betrachten. Nach SORSBY und Mitarb. [11] schwanken die Normalwerte zwischen +4,0 D und —4,0 D, nach DASCHEWSKIJ [1] zwischen +2,0 D und —2,0 D. Unsere in der Gemeinde Újléta ermittelten durchschnittlichen Refraktionswerte veranschaulichen Abb. 3 (0—15jährige Kinder) und Abb. 4 (Gesamtbevölkerung). Wie ersichtlich, bewegt sich die Kurve ober- bzw. unterhalb der durchschnittlichen Dioptriewerte in einer Breite von 1,5 D (dies

bedeutet sowohl in positiver, als auch in negativer Richtung Abweichungen von 0,75 D), woraus folgt, daß zwischen 10—59 Jahren die durchschnittliche Refraktion fast identisch ist bzw. um den Mittelwert schwankt. Der tatsächliche Wert ist aber selbstverständlich die Funktion des Alters. Die in der Altersgruppe von 5—9 Jahren (durchschnittliche Refraktion +2,2 D) vorgefundenen Werte von +1,5—3,0 D sind z. B. Streuungsametropien. Die klein- und mittelgradige Hypermetropie und Myopie sind — mit Ausnahme der im 2.—3. Lebensjahr stabilisierten relativen Übersichtigkeit und der mit Fundusveränderung einhergehenden Kurzsichtigkeit — nicht als pathologische Zustände zu betrachten. Bei den Einwohnern von Újléta waren folgende, als pathologisch auffaßbare Veränderungen vorzufinden: hochgradige Hypermetropie (2,8%), maligne Myopie (2,2%), vorzeitig stabilisierte relative Hypermetropie (7—10%), mit Fundusveränderung einhergehende Myopie mittleren Grades, sowie Anisometropie und Astigmie. Im Zustandekommen dieser Veränderungen, die in insgesamt 12—15% der untersuchten Fälle vorlagen, spielte die Erblichkeit eine bedeutende Rolle.

Aus Abb. 3 und 4 geht hervor, daß die Emmetropisation, mit Ausnahme der pathologischen Fälle, etwa im Alter von 10—12 Jahren beendet ist. Bei den 15—24jährigen Personen macht die Anzahl der Emmetropen bereits mehr als 50% aus (Tab. 4). In diesem Alter beträgt die durchschnittliche Refraktion — die bis zum 59. Lebensjahr sozusagen unverändert bleibt — bereits weniger als +1,0 D (Abb. 4). Demnach folgt bis zum Alter von 70 Jahren, wahrscheinlich wegen der Linsenveränderung und Tonusverminderung des M. ciliaris eine Hypermetropisationsphase. Während nach MARKBREITER [5] die Ursache dieser Erscheinung in der Homogenisation des Kerns und der Rinde liegt, hält WESSELY [16] die durch die altersbedingte Veränderung der Sklerastruktur verursachte Verkleinerung des Bulbus und MAIONE [4] die Verflachung der Linse für verantwortlich. Nach dem 70. Lebensjahr tritt im allgemeinen eine, durch die senile Veränderung der Linsenbrechkraft verursachte Myopisation auf.

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SCHOCK UND LUNGENÖDEM

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Die Beobachtung, daß verschiedene Schockformen eine hemmen de Wirkung auf die Entwicklung des Lungenödems ausüben können, wurde klinisch und tierexperimentell beleuchtet. Es wurde an Hand von zwei Krankengeschichten demonstriert, daß sich das Lungenödem nach Auftreten eines Schocks völlig zurückbilden kann. Des weiteren wurde an einem 3. Fall gezeigt, daß die Reihenfolge der Krankheitserscheinungen auch eine umgekehrte sein kann: das akute Lungenödem trat nach der erfolgreichen Bekämpfung des Schocks auf. Aus Tierversuchen an 106 Ratten ging hervor, daß das Zustandekommen eines experimentellen Lungenödems nach NH_4Cl-Einspritzung bzw. Einatmung von hyperbarem Sauerstoff durch einen Formalinschock gehemmt werden konnte.

Zwischen Schock und Lungenödem bestehen verschiedene Wechselbeziehungen.

1. *Entwicklung eines Lungenödems im Schockzustand.* Ein akutes Lungenödem ist im Schockzustand ziemlich selten zu beobachten. Kommt es jedoch vor, so ist es außer der Änderung der Osmolarität des Blutplasmas und der dynamischen Insuffizienz des pulmonalen Lymphkreislaufes, auf eine Hypoxie zurückzuführen. Die Lungenkapillaren sind der Hypoxie gegenüber äußerst empfindlich (WARREN und DRINKER 1942; DRINKER 1945; RUSZNYÁK, FÖLDI und SZABÓ 1957): die Hypoxie ruft eine Permeabilitätssteigerung der Wand der Lungenkapillaren hervor.

MOON und MORGAN (1936) konnten bei verschiedenen experimentellen Schockformen mit verzögerten Verlauf ein terminales Lungenödem nachweisen. Auch kriegsmedizinische Aufzeichnungen erwähnen die Entwicklung eines Lungenödems beim Schockzustand, nach Schußverletzungen. LONG (1840), BARDEN (1897) und PACK (1926) machten bei schweren Brandwunden ähnliche Beobachtungen.

Ein Herzinfarkt mit schwerem Linksversagen kann gleichzeitig Schock und Lungenödem verursachen; eine zum Schock führende Lungenembolie ruft, wenn auch selten, ein reflektorisches Lungenödem hervor.

Ein Lungenödem kann im Schockzustand auch durch die Schockbehandlung, so z. B. die Belastung des kleinen Kreislaufs mit überdosierter Infusionstherapie oder die wiederholte Anwendung von Sympathycomimetica ausgelöst werden. Die lungenödemerregende Wirkung von hohen Adrenalindosen ist seit Jahrzehnten bekannt (LUISADA 1928).

2. *Durch Lungenödem hervorgerufener Schockzustand.* Ein schweres Lungenödem kann zum Schock führen; es sammelt sich eine große Menge von

Blutplasma in den interstitiellen Gewebsspalten der Lunge an oder tritt in die Alveolen über. Mehr als 1000 ml kann auf diese Weise dem Kreislauf verloren gehen. Zum Schock kommt es also, wenn bei entsprechender Dauer und Schwere des Lungenödems der rasche Verlust großer Flüssigkeitsmengen eine Bluteindickung und Viskositätssteigerung ausgelöst hat (LUTZ und SCHUMACHER 1965). Der Schock kann beim Lungenödem auch durch die angewandte Therapie ausgelöst werden, z. B. fördert Morphin die Kollapstendenz. Aderlaß oder unblutige Stauungsligatur der Extremitäten können ebenfalls einen Schock hervorrufen. Diese Maßnahmen wirken — durch die Verminderung des Blutstroms zum Herzen und zur Lunge — beim Lungenödem günstig; anderseits kann der so bewirkte Abfall des Minutenvolumens — falls der Patient schon oligämisch und sein Minutenvolumen klein ist — einen Schock auslösen (ALTSCHULE 1956).

3. Der dritte, bis jetzt nicht beachtete und in der Literatur kaum erwähnte Zusammenhang zwischen Schock und Lungenödem ist *die Rückbildung des Lungenödems nach der Entwicklung des Schocks*. Bezüglich dieser Erscheinung soll im folgenden über einige klinischen Beobachtungen und Tierversuche berichtet werden.

In den letzten Jahren haben wir wiederholt die Erfahrung gemacht, daß das durch Linksinsuffizienz oder durch die Schädigung des Zentralnervensystems verursachte Lungenödem nach der Entwicklung des Schocks häufig aufhört.

Kasuistik

Fall 1. Der 61jährige Mann A. B. wurde wegen Mitralstenose und Herzinsuffizienz schon früher an unserer Klinik behandelt. Er erlitt vor 4 Jahren einen Lungeninfarkt, vor 2 Jahren eine Endokarditis lenta, und wurde wegen plötzlich auftretender Hemplegie mit Bewußtlosigkeit eingeliefert.

Beim mittelmäßig ernährten Patienten zeigen sich weder Zyanose noch Ödeme. Lungen ohne Befund. Die Herzdämpfung überschreitet nach links die Medioklavikularlinie um eine Fingerbreite. Mitralöffnungston, diastolisches Geräusch und akzentuierter I. Spitzenton. Absolute Arrhythmie. Herzfrequenz 100 pro min. Blutdruck 170/90 mm Hg. Die plötzliche linksseitige Hemplegie, die motorisch-sensorische Aphasie, das rechtsseitige Babinski-Symptom weisen auf eine Embolie der linken Art. carotis interna hin.

Nach vorübergehender Besserung des Zustandes trat am 4. Behandlungstag ein akutes Lungenödem auf. Zyanose, Lungen- und Trachealrasseln, Orthopnoe. Morphin s. c., Procain (10 ml 1%-ige Lösung i. v.), Sauerstoffbeatmung, Strophanthin, wiederholte Absaugung des endotrachealen Sekrets blieben erfolglos. Tachyarrhythmie von 160 pro min.

Nach 15stündigem Bestehen des Lungenödems plötzliche Blutdrucksenkung. Ein Schockzustand entwickelte sich, der Blutdruck wurde in einigen Minuten unmeßbar und gleichzeitig verschwanden die Rasselgeräusche, hörte das Lungenödem völlig auf und kehrte nie wieder zurück. 5 Stunden später trat Kammerflimmern und Lähmung der vitalen Zentren ein. Auch autoptisch war kein Lungenödem nachweisbar.

Fall 2. H. J. 81jährige Frau. In der Anamnese kommen seit Jahren wiederkehrende Extremitätenparesen mit Schwindelgefühl und spontaner Heilung vor. Patientin wurde wegen langsam, stufenweise, innerhalb von zwei Tagen auftretender Aphasie und rechtsseitiger Hemiplegie bewußtlos eingewiesen.

Bei der Aufnahme der soporösen Frau fanden sich über der ganzen Lunge mittel- bis großblasige Rasselgeräusche. Keine Zyanose oder Ödeme. Das Zwerchfell bewegte sich träge. Herz 1 Querfinger nach links vergrößert; laute Herztöne. Pulsfrequenz 120/min. Blutdruck

140/90 mm Hg. Nervensystem: rechtsseitige Hemiplegie, linksseitige Hemiparese. Augenhintergrund: starre, sklerotische Gefäße.

Unter den Laboratoriumsbefunden sind eine leichte Albuminurie und gesteigerte Urobilinogenurie bemerkenswert. Erythrozyten: 5 200 000, Leukozyten 17 500. Hb. 100% (Exsikkose). Reststickstoff 46 mg%. Das EKG zeigte eine Sinustachykardie (Frequenz 120 pro min.). Die gesenkten ST Zwischenstücke in Ableitung I und II weisen auf eine Überlastung der linken Kammer hin.

Das Lungenödem war mit dem gewöhnlichen therapeutischen Verfahren nicht zu beeinflussen, die Rasselgeräusche wurden intensiver und waren auch ohne Stethoskop zu hören. In 12 Stunden entwickelte sich eine zentrale und periphere Insuffizienz. Als der systolische Blutdruck unter 50 mm Hg sank, hörte das Lungenödem völlig auf und kehrte nicht wieder zurück. Patientin starb in der 17. Stunde der Klinikbehandlung.

Autoptische Diagnose: Emollitiones multiplices et cicatrices cerebri, hydrocephalus internus, arteriosclerosis generalisata, hypertrophia et dilatatio ventriculorum cordis. In der Lunge konnte man kein Ödem nachweisen.

Fall 3. Bei dem 54jährigen Patienten G. M. mit Linksversagen war die Reihenfolge der Krankheitserscheinungen eine umgekehrte: ein akutes Lungenödem trat nach der erfolgreichen Bekämpfung des Schockzustandes auf.

Patient machte vor 4 und 3 Jahren wiederholt Herzinfarkte durch, die späteren Beschwerden waren auf ein Aneurysma der linken Kammer und auf eine Herzinsuffizienz zurückzuführen. Wegen Herzinsuffizienz wurde er in den letzten 2 Jahren viermal behandelt.

2 Monate nach dem letzten Klinikaufenthalt traten abends starke pektanginöse Beschwerden mit Schwitzen auf. Die Schmerzen hörten erst nach Einspritzung von Pethidin auf. Der Kranke wurde mit der Diagnose eines rezidivierenden Myokardinfarkts wieder eingewiesen.

Klinikaufnahme im Schockzustand. Die schweißbedeckte Haut war blaß und zyanotisch. Kühle Extremitäten. Lungen o. B. Herz 2 1/2 Querfinger nach links vergrößert. Ein früher nicht hörbares apikales, holosystolisches Geräusch wies auf eine Mitralinsuffizienz hin. Pulsfrequenz 96/min. Blutdruck 80/70 mm Hg. Leber um 5 Querfinger vergrößert.

Auch die Laboratoriumsbefunde sprachen für einen rezidivierenden Myokardinfarkt. SGOT 880 Einheiten. Rote Blutkörperchen 4,3 Millionen, weiße 20 400. Hb 83%. BSR: 17 mm. Das EKG zeigte einen Tawara-Schenkel-Block und im Vergleich mit den früheren EKG-Aufnahmen eine wesentliche Änderung.

Die Schockbehandlung erwies sich anfangs als wirkungslos, der Blutdruck sank und wurde unmeßbar, die Urinausscheidung hörte völlig auf. Pulslosigkeit. Lungen: noch immer o. B., kein Lungenödem, die Atmung sehr frequent, aber keine Atemgeräusche.

Als es aber endlich gelang, den Schock zu beseitigen, und der Blutdruck auf 90/70 mm Hg, später auf 120/80 mm Hg anstieg, erschien alsbald ein akutes Lungenödem. Das Rasseln verstärkte sich und war weithin hörbar. Der Patient hustete eine große Menge von dünnem, serösem, leicht rosa gefärbten Sputum aus. Das Lungenödem zeigte nach Verabreichung von Morphin, Strophanthin, endotrachealer Sekretabsaugung und Sauerstoffinhalation keine wesentliche Besserung. Am zweiten Behandlungstag entwickelte sich ein paralytischer Ileus und der Patient ging infolge einer plötzlichen starken Hämatemesis ad exitum.

Sektionsbefund: Infarctus recens et priscus myocardii, aneurysma ventriculi sinistri cordis, erosio haemorrhagica ventriculi et intestini, oedema pulmonum mai. grad. Die frische Hinterwandnekrose der linken Kammer war sehr ausgedehnt, die Lungen waren hochgradge mit Ödemflüssigkeit durchtränkt.

Tierversuche

Methodisches. Die Tierversuche wurden an 106 Wistar-Ratten von etwa 200 g Körpergewicht ausgeführt. Akutes Lungenödem wurde durch eine intraperitoneale Injektion von Ammoniumchlorid (0,7 ml 100 g 6%ig) bzw. durch Einatmung von reinem hyperbaren Sauerstoff (3,7 Atü 3 Stunden hindurch) in einer 60 l Überdruckkammer hervorgerufen. Bezüglich der Einzelheiten der Methodik soll auf frühere Mitteilungen hingewiesen werden (König und König 1949, Gottsegen, Szám und Csornay 1954, 1956, Szám, Nikolits und Gottsegen 1966).

Das Ausmaß des Lungenödems wurde durch Bestimmung des relativen Lungengewichtes sowie durch histologische Untersuchungen festgestellt. Die Auswertung der mikroskopischen Befunde erfolgte ohne Kenntnis der experimentellen Daten. Mit +++ wurden sich auf die gesamten Lungenlappen, mit ++ auf einzelne Lappen erstreckende, mit + herdweise zerstreute Veränderungen bezeichnet. Die Schwere des Ödems wurde in den einzelnen Serien

durch Vergleich einerseits der histologischen Befunde, anderseits der Mittelwerte der Lungen-Körpergewichtsindices (relatives Lungengewicht = Lungengewicht × 100/Körpergewicht) beurteilt. Der normale Wert des relativen Lungengewichtes erwies sich bei 9 normalen Ratten 0,84 ± 0,04.

Der Schockzustand wurde durch Formalin hervorgerufen. Die mit Formalin vorbehandelten Tiere erhielten 3mal 0,5 ml einer 4%igen Lösung s. c. innerhalb von 20 Std. und 1 Std. nach der letzten Injektion wurde Ammoniumchlorid injiziert.

Die Körpertemperatur der Tiere wurde rektal gemessen. Der Sauerstoffverbrauch der nüchternen Tiere wurde nach ISSEKUTZ u. ISSEKUTZ 1942 in je 10 Meßperioden zu 5 min. vor der ersten und 4 Std. nach der letzten Formalininjektion bestimmt. Zum Vergleich wurden die arithmetischen Mittelwerte der beiden Bestimmungsreihen zugezogen. Der Blutdruck wurde teils unblutig mittels des photoelektrischen Manometers von HERR an der Vorderpfote, teils auf blutigem Wege in der frei präparierten Femoralis mit einem Elema-Elektromanometer gemessen. Die statistische Auswertung der Ergebnisse erfolgte mittels der Studentschen T-Probe.

Versuchsergebnisse

1. In der ersten Versuchsreihe wurde die Wirkung des Formalin-Schocks an 44 Wistar-Ratten geprüft. Akutes Lungenödem wurde bei 21 Versuchstieren durch Einatmung von hyperbarem Sauerstoff, bei weiteren 23 durch Einspritzung von NH_4Cl hervorgerufen. 21 Tiere erhielten Formalininjektionen, die übrigen dienten als Kontrolle. Die Versuchsergebnisse sind aus Tabelle I ersichtlich. Die histologische Untersuchung der Lunge und die relativen Lungengewichte zeigten in den beiden Lungenödemformen zwischen Formalinschock-Tieren und Kontrollgruppe einen signifikanten Unterschied.

Tabelle I

Die protektive Wirkung der Formalinvorbehandlung bei experimentellem Lungenödem, hervorgerufen durch NH_4Cl bzw. durch Einatmung von hyperbarem Sauerstoff

(3 Stunden, 3,7 Atü.)

Experiment		Grad des Lungenödems (histologisch)				Durchschnittl. Überlebensdauer (Min.)	Relat. Lungengewicht $\left(\frac{100 \times \text{Lungengewicht}}{\text{Körpergewicht}}\right)$	Zahl der Überlebenden
		0	+	++	+++			
Hyperbares Lungenödem	Formalin-Schock	6	2	3	0	243	1,23 ± 0,03	1
	Kontrolle	2	1	1	6	237	1,64 ± 0,04	2
Salmiak Lungenödem	Formalin-Schock	8	2	∅	∅	30	0,86 ± 0,03	2
	Kontrolle	1	6	4	2	34,5	1,3 ± 0,03	1

a) Hyperbares Lungenödem. Während das hohe durchschnittliche relative Lungengewicht (1,64 ± 0,04) der Kontrolltiere auf hochgradiges Lungenödem hinwies, war das relative Lungengewicht bei den formalinbehandelten Tieren

nur mäßig erhöht (1,23 ± 0,03). Die histologische Untersuchung zeigte kein hochgradiges und 5 mittelgradige Lungenödeme in der Formalingruppe, 6 Tiere blieben ödemfrei. Demgegenüber waren in der Kontrollgruppe 6 hochgradige und 2 mittelgradige Ödeme nachzuweisen, 2 waren ödemfrei.

b) Salmiak Lungenödem. Die Ergebnisse waren ähnlich wie bei der früheren Gruppe. In der Kontrollgruppe war das Lungengewicht 1,3 ± 0,03, gegenüber dem völlig normalen Wert (0,86 ± 0,03) der Formalin-Schocktiere. Histologisch waren keine hochgradigen und nur 2 geringfügige Ödeme nachzuweisen, die Anzahl der ödemfreien Tiere betrug 8. In der Kontrollgruppe kamen 2 hochgradige und 10 mittelgradige Lungenödeme vor und nur ein einziges Tier war frei von Ödem.

Tabelle II

Körpertemperatur, Grundumsatz und Blutdruck nach Formalinverabreichung

Der Sauerstoffverbrauch wurde an denselben Tieren vor und nach dem Versuch bestimmt; zu den übrigen Versuchen wurde stets eine besondere Kontrollgruppe zugezogen

	Temperatur (°C)	O_2-Verbrauch ml/100 g/5 min	Syst. Blutdruck unblutig mm Hg	Syst. Blutdruck blutig mm Hg
Kontrolltiere	38,2 ± 0,25	21,1 ± 0,83	109 ± 2,76	99 ± 4,12
Formalin-Schock-Tiere	36,0 ± 0,35	16,9 ± 1,01	65 ± 4,62	50,7 ± 3,95

Die durchschnittliche Überlebensdauer und die Anzahl der Überlebenden zeigte bei beiden Lungenödemformen keinen signifikanten Unterschied.

2. In einer zweiten Versuchsreihe wurden 21 mit Formalin vorbehandelte und 20 Kontrolltiere bezüglich des Verhaltens ihrer Körpertemperatur miteinander verglichen. 2 Std. nach der letzten Formalininjektion war die Temperatur der vorbehandelten Tiere durchschnittlich 2,2 °C niedriger als die der Kontrollen (Tab. II). Die Abweichung erwies sich als stark signifikant ($t = 5{,}184$, $P < 0{,}01$). Förderung der Wärmeabgabe durch einen Ventilator am Käfig hat in keiner der beiden Gruppen nennenswerte weitere Änderungen der Temperatur bewirkt: sie sank durchschnittlich um 0,15 °C bei den Formalin-, um 0.28 °C bei den Kontrolltieren.

3. In der nächsten Versuchsreihe wurden die formalinbehandelten Tiere durch Heizung des Käfigs auf 38 °C erwärmt; dies wurde bei einer Umgebungstemperatur von etwa 40 °C binnen 20 min erreicht. Nun wurde diesen Tieren — die nach der Erwärmung wesentlich lebhafter waren — sowie der bei Zimmertemperatur gehaltenen Kontrollgruppe Ammoniumchlorid injiziert.

Die histologischen Befunde und relativen Lungengewichte von 10 Versuchs- und 11 Kontrolltieren sind in Tab. III zusammengefaßt. Die Versuchstiere sind 20—45 (durchschnittlich 28), die Kontrollen 20—60 (durchschnittlich 38) min nach der Injektion eingegangen; die innerhalb von 20 min gestorbenen

Tabelle III

Formalinschock wehrt das Salmiak-Lungenödem auch bei konstanter Körpertemperatur ab

	Lungenödem				Überlebend	Relat. Lungengewicht
	+++	++	+	0		
Ammoniumchlorid	6	1	3	1	—	1,64 ± 0,128
Ammoniumchlorid + Formalin-Schock + Erwärmen	—	1	1	8	—	1,02 ± 0,054

Tiere blieben unberücksichtigt, da nach unseren früheren Erfahrungen zur Entwicklung des Salmiak-Lungenödems 20 min nötig sind. Es gab keine Überlebenden. Die meisten Formalintiere blieben ödemfrei, während das histologische Bild der Kontrolltiere — mit einer einzigen Ausnahme — stets Lungenödem, zumeist schweren oder mittleren Grades, aufwies. Dementsprechend war das relative Lungengewicht der Kontrollen stark erhöht, das der Versuchsgruppe hingegen nahezu normal. Der Sauerstoffverbrauch wurde bei jedem Tiere vor und nach den Formalininjektionen bestimmt. Tab. II enthält die Mittelwerte der Bestimmungen sowie die Änderung der Ausgangswerte. Bei allen Tieren sank der Ruheumsatz um 8—46% (durchschnittlich 20%). Die Abweichung erwies sich als stark signifikant ($t = 9{,}1$, $P < 0{,}01$). Die intraarteriellen Blutdruckwerte waren um 10—15 mm Hg niedriger als die auf unblutigem Wege erhaltenen, doch wurde mit beiden Methoden ein Druckabfall von über 40 mm Hg nach der letzten Formalineinspritzung festgestellt (Tab. II). Die Abweichung zwischen Versuchs- und Kontrollgruppen erwies sich als stark signifikant ($t = 10{,}75$ bei blutiger, 8,244 bei unblutiger Messung, $P < 0{,}01$ für beide Gruppen). Das Körpergewicht wurde nach 24stündigem Nahrungs- und Wasserentzug untersucht. Es sank um 10,4 g bei den unbehandelten, um 7,8 g bei den Formalintieren; der Unterschied war nicht signifikant.

Besprechung

Der Mechanismus, durch welchen der Schockzustand das akute Lungenödem behebt, besteht insbesondere in der Abnahme des venösen Rückflusses, wodurch die rechte Kammer und der kleine Kreislauf entlastet werden. Es entwickelt sich eine einfache oder eine mit Hämokonzentration einhergehende Hypovolämie (Wollheim 1967). Bei schwerem Schock führt auch der Plasmaaustritt aus den Gefäßen zur verminderten Füllung der rechten Kammer und des kleinen Kreislaufs bzw. zur Abnahme des Minutenvolumens, die ihrerseits eine Verminderung des peripheren Druckes zur Folge hat. Eine weitere Erscheinung ist die Erweiterung des Kapillarnetzes: das Blut in der Kapillarbahn strömt langsam oder kann sogar stagnieren. Im irreversiblen Stadium

kommt noch eine allgemeine, periphere Vasodilatation dazu. Diese hämodynamischen Effekte des Schocks erklären die Tatsache, daß viele Formen von akutem Lungenödem nach dem Auftreten des Schocks verschwinden. SARNOFF und FARR (1944) behandelten das Lungenödem durch mittels spinaler Anästhesie hervorgerufene Blutdrucksenkung und erklärten die günstige Wirkung mit der Verminderung des Blutrückflusses zum rechten Herzen. LITARCZEK (1958) stellte ebenfalls die günstige Wirkung der künstlich hervorgerufenen Blutdrucksenkung bei Patienten mit Lungenödem fest. Er beobachtete, daß bei Patienten, bei denen er eine größere Blutdrucksenkung erzielen konnte, das Lungenödem schneller verschwand als dort, wo der Blutdruck nur wenig abfiel. Auch SARNOFF, BERGLUND und SARNOFF (1953) haben bemerkt, daß im irreversiblen Schock eine beträchtliche Blutmenge aus dem kleinen in den großen Kreislauf geleitet werden kann.

Auf Grund theoretischer Erwägungen muß man auch mit einem Druckabfall in den Pulmonalgefäßen rechnen. Der Filtrationsdruck der Lungenkapillaren ist vom Gesichtspunkt des Zustandekommens der alveolären Transsudation einer der wichtigsten Faktoren. Tierversuche haben aber gezeigt, daß bei zahlreichen experimentellen Schockformen die Druckverhältnisse des kleinen Kreislaufs bis zum Endstadium unverändert bleiben (HALMÁGYI 1957), oder die Druckwerte nur wenig abnehmen (TAKÁCS, NAGY und KÁLLAY 1957). Es entwickelt sich im kleinen Kreislauf eine Vasokonstriktion infolge der Anoxie (EULER und LILJENSTRAND 1946; TAKÁCS 1963) mit der Zunahme der pulmonalen-vaskulären Resistenz; der Druck in den Lungenkapillaren zeigt deswegen keine oder nur eine mäßige Abnahme. Diese Abnahme ist bei einem durch Hämorrhagie oder Trauma hervorgerufenen Schock am ausgeprägtesten (TAKÁCS 1963). Die Drucksenkung in den Lungenkapillaren ist also für die Rückbildung des Lungenödems nach der Entwicklung des Schocks höchstens teilweise verantwortlich. Der Flüssigkeitsgehalt der Lunge ist aber nicht nur von den Druckverhältnissen, sondern auch von der pulmonalen Durchströmung abhängig. Die Hypoxie verursacht während des Schocks eine Vasokonstriktion in der Lunge (EULER und LILJENSTRAND 1946), die periphere Resistenz nimmt zu, die Druckwerte des kleinen Kreislaufs bleiben unverändert oder zeigen nur eine geringe Abnahme, der Blutgehalt der Lunge nimmt aber wesentlich ab. Dadurch ist auch der Anstieg der Vitalkapazität nach Aderlaß bei Asthma cardiale zu erklären: mit der Abnahme des Blutgehalts der Lunge nimmt deren Luftgehalt (die Vitalkapazität) zu. Im Schockzustand entsteht eine Art unblutiger »Aderlaß« in die peripheren Gefäße. Die Folge ist eine Verminderung des Herzminutenvolumens und damit auch der Füllung im kleinen Kreislauf. Auch andere Prozesse können eine Rolle spielen. Wegen der generalisierten Flüssigkeitsausströmung in den extravasalen Raum wird die Flüssigkeitsausströmung in der Lunge geringer und das schon entwickelte Transsudat wird durch die Lymphgefäße entfernt.

In früheren Versuchen (GOTTSEGEN, SZÁM und CSORNAY 1957) ist die Feststellung von SELYE (1938), wonach die Auslösung einer Alarmreaktion durch Formalineinspritzung die Tiere vor Lungenödem schützt, vollauf bestätigt worden, und zwar nicht nur beim Adrenalin- und Kochsalzinfusionsödem, sondern auch beim hyperoxischen und Salmiaködem. Doch konnten wir der Schlußfolgerung, daß dieser Schutzeffekt auf einer vermehrten Produktion von Glucocorticoiden beruhe (Selye 1950), nicht beipflichten. Cortisonverabreichung hemmte in unseren Versuchen die Entstehung von Lungenödem nicht, auch wurde es durch Desoxycorticosteron nicht gefördert. Wir fanden, daß Formalinverabreichung auch bei adrenalektomierten Tieren eine volle Schutzwirkung ausübt. Die Annahme einer Streßreaktion mit Hyperaktivität der Nebennierenrinde muß demnach widerlegt werden.

Der Nachweis einer unveränderten Schutzwirkung des Formalins auch nach Adrenektomie hat unser Augenmerk in erster Linie auf den Kreislaufeffekt dieser Vorbehandlung gelenkt. KÖNIG und KÖNIG (1949), die bei 3 Ratten Hemmung des Salmiaködems nach Formalin beobachtet haben, schrieben dies dem Schockzustand der Tiere zu, der die Resorption des Salmiaks aus der Bauchhöhle verhindert haben sollte. Die Unrichtigkeit dieser Auffassung beweist die gegenüber der Kontrollen unbeeinflußte Letalität und sogar verkürzte Lebensdauer unserer vorbehandelten Tiere. Die unmittelbare Beobachtung der somnolenten Formalintiere veranlaßte uns, an der ursächlichen Bedeutung einer akuten hämodynamischen und Stoffwechselschädigung festzuhalten und deren Wirkung auf die Hemmung des Ödems zu analysieren.

Die Herabsetzung der Körpertemperatur im schweren Schock bildet einen nicht zu vernachlässigenden Faktor. Die Schutzwirkung der Unterkühlung wurde bei experimentellem Lungenödem von CAMPBELL (1937), GROSSMANN und PENROD (1949) und KÖNIG und KÖNIG (1949) nachgewiesen; Hyperthermie erhöhte hingegen die Ödembereitschaft (HADDY, CAMPBELL und VISSCHER 1949). Die Rektaltemperatur unserer Tiere sank nach Formalin. Die Schutzwirkung blieb jedoch in beiden Versuchsreihen erhalten, auch wenn der Kühleffekt durch Erhöhung der Umgebungstemperatur kompensiert wurde; dieser dürfte zumindest nicht allein für die Verhütung des Lungenödems verantwortlich gemacht werden.

Aus früheren Untersuchungen (GOTTSEGEN, ROMODA und BARTOK (1954), GOTTSEGEN, KOHEN und ROMODA (1956) ist bekannt, daß gegen Lungenödem wirksame Medikamente, wie Morphium und Procain, den Grundumsatz herabsetzen. Die in unseren Versuchen gefundene Senkung des Ruheumsatzes um durchschnittlich 26% nach den Formalininjektionen dürfte zur Hemmung der Ödementstehung wesentlich beitragen. Die auch nach Adrenektomie erhaltene Schutzwirkung einer Formalinvorbehandlung ist also eine Folge des bestehenden Schockzustandes. Der bei den mit Formalin behandelten Tieren beobachtete Blutdruckabfall von durchschnittlich 40 mm Hg ist geeignet, die wich-

tigste hämodynamische Voraussetzung des Lungenödems, die Blutanschoppung im kleinen Kreislauf, weitgehend zu beeinflussen. Dieser Mechanismus, der auch der ödemhemmenden Wirkung der Vasodilatatoren und Ganglionblocker (SARNOFF, GOODALE, SARNOFF 1952, HILDEN 1953, DAVIES und Mitarb. 1953, STORSTEIN und TVETEN 1954, KOCH 1955, FERSINI und SUSCA 1955, ELLESTAD und OLSON 1956, KELLNER 1957 usw.), sowie der Rückenmarkanästhesie (SARNOFF und FARR 1944) zugrunde liegt, wird auch im experimentellen Formalin-Schock in Gang gesetzt.

Für die Hilfe in der histologischen Untersuchung sowie in den unblutigen Blutdruck- bzw. Grundumsatzbestimmungen sagt Verfasser Frau Dr. med. M. Csornay, Herrn Dr. med. habil. T. Gáti und Frau Dr. med. M. Istvánffy auch auf diesem Wege besten Dank.

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DIABETES MELLITUS IN PITUITARY INSUFFICIENCY

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In a 65-year-old male the sudden onset of diabetes was witnessed 15 years after hypophysectomy for a chromophobe adenoma. Two and a half years later, the patient developed hepatic cirrhosis and died in consequence of staphylococcal enterocolitis and pneumonia. Autopsy revealed a pseudocyst with scarce pituitary tissue at the site of the removed gland. Response to methyrapone and the presence of lipid-mobilizing factor pointed to some preserved pituitary activity. This case makes it doubtful whether the pituitary gland is necessarily involved in the pathogenesis of diabetes.

The spontaneous occurrence of pituitary failure together with diabetes is very rare. By the increasing practice of hypophysectomy for diabetic angiopathy it has become possible to study HOUSSAY's phenomenon in the human subject. Pituitary failure preceeding the onset of diabetes has been reported in six cases only. Any observation of this kind may raise interesting points concerning the pathogenesis of diabetes.

Case report

A. L., a 50-year-old male patient had been subjected to hypophysectomy for a chromophobe adenoma in 1946. The operation had been followed by weakness, drowsiness, easy fatigue, dryness of the skin, puffiness of the eyelids, loss of body hair, slow speech, loss of potency, and constipation. The patient was treated at first with ACTH and DOCA, then with 12.5 to 25 mg of cortisone and 100 mg of thyroid extract daily. This kept him in a satisfactory condition, only three to six times a year in the course of certain episodes such as influenza, pneumonia or heavy purgation he would become suddenly drowsy, difficult to arouse from sleep, or even comatose. These bouts which were accompanied by temperatures rising to 40 °C even in the absence of any apparent infection and by a drop of blood pressure to 80 or 90 mm Hg, promptly responded to an intramuscular injection of 150 mg of cortisone.

Laboratory investigations. Urinary ketosteroid output was between 4.6 and 7.8 mg, exceptionally between 10 and 12 mg daily, that of ketogenic steroids (by the method of NORIMBERSKY) between 0 and 7 mg, with occasional peaks of 13 mg. 40 U. Zn-ACTH elicited a rise in the excretion of 17-ketosteroids

from 4.6 mg to 11.4 mg, leaving unchanged that of ketogenic steroids. Methyrapone caused a reduction in the excretion of 17-ketosteroids and a 5.8 mg rise in that of ketogenic steroids. (By our method, the lowest limit of normal increase is 8 mg.) The plasma hydrocortisone level varied between 0 and 4 μg per

Fig. 1. Cystic structure occupying the site of the removed pituitary gland

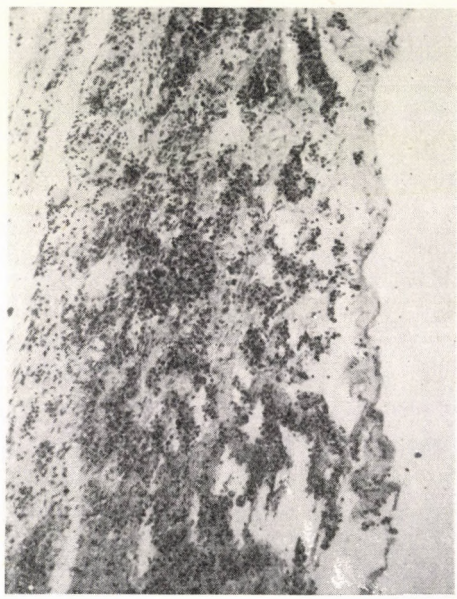

Fig. 2. Adeno-pituitary cell groups in the wall of the cyst occupying the sella

100 ml. Urinary aldosterone output was 3.8 μg daily. Basal metabolic rate was between —7 and —12 per cent. The thymol precipitation test was 4.8 units, the gold sol reaction negative. Serum bilirubin was 0.7 mg per 100 ml. Effect of protein-loading on blood sugar, 122—56—104 mg per 100 ml. (As demonstrated by GÓTH in 1952, a reduction of the blood sugar level after protein intake is a sign of pituitary failure.) Blood sugar tolerance curve with dextrose: 86—110—104—90—80 mg per 100 ml. Serum potassium, between 4.0 and 5.0 mEq. Serum sodium, between 139 and 140 mEq. Endogenous creatinine clearance, 103 ml. Erythrocytes, between 3,700,000 and 4,400,000; leucocytes, 6400; haemoglobin, 12 g per 100 ml; serum iron, 70 μg per 100 ml, serum cholesterol, 160 mg per 100 ml.

Apart from the foregoing episodes the patient had been fit during 15 years. Then he suddenly experienced excessive thirst compelling him to drink 3 l water daily and he lost 1.5 kg in four days. Blood sugar was 313 mg per

100 ml in the morning, 296 at midday and 440 in the evening, and the urinary excretion of sugar was 77 g daily. Reduction of dietary carbohydrates to 180 g daily was followed by a fall in sugar excretion to 20 or 30 g. Carbutamide was prescribed in initial dose of 3 g. The diabetes improved, permitting a successive

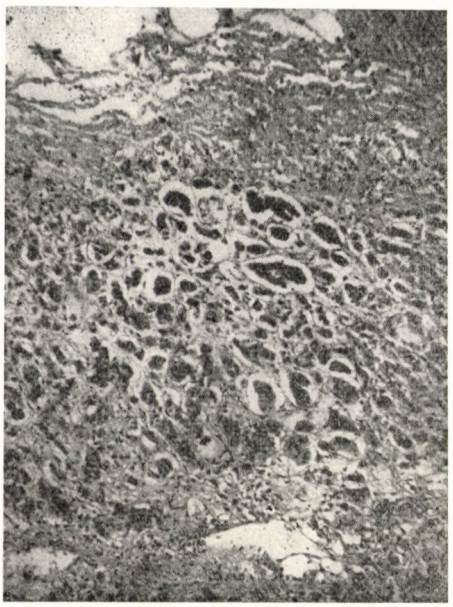

Fig. 3. Adrenal cortex with signs of atrophy and a disorganized cellular pattern. The zone fasciculate is conspicuously narrow

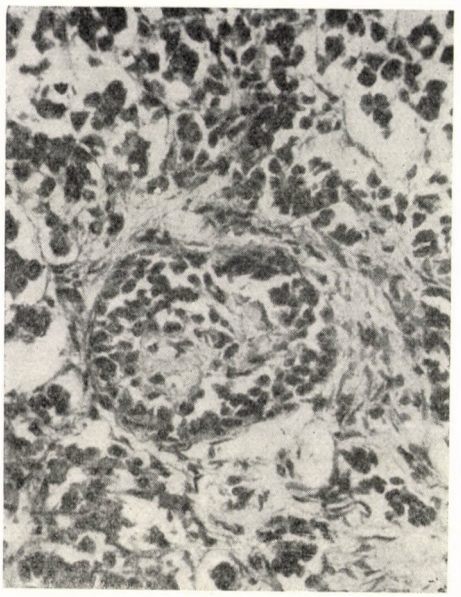

Fig. 4. Langerhans's islet with hyalinized centre

reduction of the dose by 0.5 g daily to a maintenance dose of 0.5 g daily on which the patient remained sugar-free, with blood sugar values of 146, 194, and 173 mg per 100 ml, respectively. The urinary fat-mobilizing factor was examined by the method of CHALMERS. The patient was kept on a diet containing 180 g carbohydrates during four days, and on a 1000 calorie and 50 g carbohydrate diet for further four days. Injection into mice of the extracts of urine eliminated during the low carbohydrate intake caused a reduction in body-weight by 2 g and an increase in the serum lipids whereas the urine obtained during massive carbohydrate intake had no effect at all.

The patient did well for another two years during which time he experienced only two episodes of drowsiness. His last admission in September 1963 was made necessary by anorexia and weakness. The blood sugar values were 166 mg per 100 ml in the morning, 279 mg per 100 ml at midday, and 300 mg per 100 ml in the evening. Glycosuria was slight. The thymol precipitation test was 14.6 units, gold sol reaction 5 units, serum bilirubin 3.0 mg per 100 ml by

the prompt direct reaction. Liver and spleen were not palpable. In a few weeks ascites developed, this was followed by a staphylococcal enterocolitis with haemorrhagic stools, accompanied by pneumonia and pulmonary oedema. Cortisone, fluid and electrolyte therapy, cardiac drugs, and sulphaguanidine were given without any benefit. The patient died in January, 1964.

Post mortem finding. The body was that of a moderately obese elderly male. On the right side of the scalp over the frontal and parietal regions there was a diagonal scar resulting from the operation 15 years earlier. Both frontal lobes contained several emollition cysts of walnut size. There was a walnut-sized structure identifiable as a cyst with a 2 to 3 mm thick fibrous capsule, protruding from the sella to the cranial basis. Its lumen was filled with a brownish-red turbid fluid. The sella was abnormally wide, its walls were brittle. The hypothalamic structures over the cyst were softened. There was a generalized atrophy of the endocrine organs, especially of the adrenal cortex, which displayed conspicuously lipid-poor areas. The other findings included generalized atherosclerosis; atrophic cirrhosis of the liver, acute catarrhal enterocolitis and focal bronchopneumonia. Microscopic findings: The wall of the cyst occupying the sella was made up of fibrous tissue with an epithelial lining. Between the fibrous strands of irregular arrangement, chronic inflammation was demonstrable with calcium and haemosiderin deposits.

In the intrasellar portion of the capsule there were smaller groups of anterior pituitary cells, mostly of the chromophobe type but by the Mallory-Crossman staining method eosinophile and basophile cells were also demonstrable in some groups. The follicles of the thyroid were marked by narrow lumina, a low epithelium, and an abundance of colloid. The testicles showed canalicular atrophy and hyaline degeneration, the majority contained no Leydig cells. The adrenal cortex, particularly the zona fasciculata, was narrow, most of the cells were poor in lipid. In the pancreas there was an excess of connective tissue and microfocal fatty necrosis. Incipient hyaline degeneration was demonstrable in a number of Langerhans's islets.

Discussion

In a male patient, hypophysectomy was followed after a 15-year interval by the sudden onset of diabetes of medium severity. Though the possibility of steroid diabetes cannot be ruled out, it is made unlikely by the fact that the patient had been on small maintenance doses (12.5 and 25 mg) of cortisone for 15 years which were continued also after the onset of diabetes without any adverse effect on this condition. In pituitary failure, even under substitution treatment with cortisone, diabetes generally remains absent. In the present case, cirrhosis of the liver appeared in the third year after the onset of diabetes,

therefore it could not have had any aetiologic significance. The foci of necrosis found in the pancreas were too small to account for the production of diabetes. The ready response to carbutamide was a further evidence of insulin secretion.

The original tumour removed 15 years earlier had been replaced by a pseudocyst bulging into the basal region of the brain with consequent destruction of almost the whole hypothalamus. The cyst showed occasional groups of eosinophile and basophile cells. These might have been preserved from the original tumour or were signs of a recurrence. Though the number of the remaining cells was very small, they must still have had some endocrine activity. Overall endocrine hypofunction was reflected by the finding of thyroid, adrenocortical and testicular atrophy. Incipient hyaline degeneration of Langerhans's islets may be regarded as a morphologic evidence of diabetes. The slight rise in corticosteroid excretion elicited by methyrapone and the presence of the lipid-mobilizing factor suggest that a modest endocrine activity was still preserved. The production of lipid-mobilizing factor is generally ascribed to the anterior pituitary lobe though the posterior lobe also contains a substance of this kind (RUDMAN et al., SEIFTER and al., LÉLEK, CHALMERS). The present authors (GÓTH et al.) also failed to demonstrate the presence of lipid-mobilizing factor after a low-calorie diet in the urine of two patients, one with Sheehan's syndrome and the other with a chromophobe adenoma.

Search for the cause of diabetes leads directly to the pituitary gland, since STH, ACTH, prolactin and to a certain extent also TSH are known to elevate the blood sugar level. YOUNG succeeded in inducing diabetes by means of STH (metahypophysial diabetes) and considers this hormone to represent a decisive factor in the pathogenesis of diabetes. In conformity with this claim, JULESZ incriminates the pituitary gland for the production of juvenile diabetes (JULESZ et al.). According to VALLANCE-OWEN et al., insulin-antagonists do not take effect in the absence of a functioning pituitary gland whereas other investigators including MAGYAR deny any participation of the pituitary gland in the production of diabetes, except for that associated with Cushing's disease or with acromegaly.

In the present case diabetes developed though very little functioning pituitary tissue had been preserved. This pleads against the view which inseparably links up the pathogenesis of diabetes with the presence of functioning pituitary tissue.

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EFFECT OF HYPERTONIC MANNITOL SOLUTION ON CIRCULATION AND RENAL FUNCTION IN ACUTE BLOOD LOSS

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In the anaesthetized dog with haemorrhagic hypotension and with the arterial pressure stabilized at 60 mm Hg, 200 ml of a 20 per cent mannitol solution significantly enhances diuresis, increases the inulin and PAH clearances, though the former does not reach the normotensive control level. Retransfusion of the lost blood further increases diuresis, but not the clearance values. In response to mannitol the directly measured renal blood flow increases moderately, renal resistance decreases slightly, by 16 per cent on the average.

When arterial pressure was kept at the same hypotensive level, mannitol significantly enhanced cardiac output and reduced peripheral vascular resistance by 33 per cent.

When further blood loss was prevented during mannitol infusion, arterial pressure rose from 60 to 89 $\pm$ 3.1 mm Hg, cardiac output increased to nearly the double of the pre-haemorrhagic value and total vascular resistance decreased to 35 per cent of the control value. Retransfusion of blood caused no further increase in cardiac output.

Diuresis also increased in the latter experiment. PAH and inulin clearances rose high above the control values, but this was due mainly to an elimination of clearance substances accumulated in the kidney during hypotension.

Mannitol increased the directly measured renal blood flow to the normotensive control level; renal vascular resistance decreased significantly (by an average of 33 per cent).

The results indicate that the decrease of vascular resistance caused by mannitol is not due exclusively to the decrease of blood viscosity, nor a specific renal effect, and it manifests itself more markedly in systemic than in renal circulation.

Hypertonic mannitol solution is known to be a potent osmotic diuretic [14]. This has gained practical importance when it was shown that the diuretic effect is exerted also in blood loss or in other hypotensive conditions. Therefore, intravenous infusion of mannitol has been recommended for the prevention and treatment of posttraumatic or postoperative oliguria or anuria [1, 10, 11, 12]. Hypertonic mannitol is, however, not only a tubular diuretic, i.e. one acting by diminishing water and electrolyte reabsorption, but it also enhances glomerular filtration rate (GFR) [11] and renal blood flow (RBF) [3, 5], and decreases renal vascular resistance. The latter is, however, due, at least in part, to a haemodilution, to a decrease of blood viscosity by the hypertonic solution. It should be realized that a decrease of viscosity may profoundly influence not only the renal, but also the systemic haemodynamics. The introduction into the circulation of considerable quantities of an osmotically active substance may significantly increase the volume of circulating plasma and, in oligaemia, secondarily also the cardiac output. To clarify these systemic haemo-

dynamic effects, in dogs with haemorrhagic hypotension we studied the effect of hypertonic mannitol infusion on cardiac output, total peripheral vascular resistance (TPR), RBF, renal vascular resistance, diuresis, and GFR.

Methods

Mongrel dogs of both sexes, with a mean weight of 18.5 (15 to 28) kg, anaesthetized with pentobarbital (0.30 mg/kg intravenously), were used. The left kidney was exposed from a median abdominal incision and a nylon tube of large diameter was ligated into the renal vein. This was connected to a cannula in the jugular vein through a silicon rubber tube system. Renal outflow was measured directly. To collect urine, polyethylene tubes were inserted in both ureters. Arterial blood pressure was measured by means of a mercury manometer ligated into the femoral artery. Mixed venous blood was obtained by means of a cardiac catheter inserted into the right atrium. Cardiac output was determined by the direct Fick method. Oxygen consumption was measured by spirometry. GFR was estimated on the basis of the inulin clearance, and renal plasma flow (RPF) on the basis of the PAH clearance and extraction.

There were two types of experiment. In one group of 17 dogs, we determined at first in two periods the parameters mentioned, then by rapid bleeding into a levelling container arterial pressure was reduced to 60 mm Hg. The tests were repeated 20 to 30 minutes after the onset of bleeding, when blood pressure had already stabilized. Then 200 ml of a 20 per cent mannitol solution was infused in 20 to 30 minutes, keeping arterial pressure at 60 mm Hg throughout. This caused a further blood loss of 290 $\pm$ 35 ml. When about half the mannitol had been infused, collection of urine and blood samples started again. Subsequently, the blood in the levelling container was retransfused in 10 to 15 minutes, and the tests were again repeated.

In the second group of 14 dogs after two control periods arterial pressure was lowered to 60 mm Hg. The tests were carried out again during hypotension. Then the levelling container was shut off and 200 ml of a 20 per cent mannitol solution was infused. Thus, in this experiment there was no further blood loss, therefore blood pressure increased during mannitol infusion. Ten minutes after the onset of mannitol infusion the tests were carried out again. Ten minutes after the infusion had been completed the blood in the container was retransfused and the tests were repeated.

In every experiment the animals received physiological NaCl solution containing 5 per cent inulin and 0.2 per cent PAH, infused at a rate of 2 ml per minute. During the hypotensive period the rate of infusion was reduced to 0.5 ml/min. Collection of urine was begun half an hour after the onset of infusion. Urine was collected in two control periods and in the hypotensive period, 20 minutes in duration each. During and after mannitol infusion the periods lasted 10 minutes each. Blood samples at mid-period were obtained from the femoral artery, right atrium and renal vein.

Oxygen saturation of arterial and venous blood was determined by means of an Atlas oxymeter, the blood and urine samples were tested for alkali-resistant inulin according to HIGHASI and PETERS [7], and for paraaminohippuric acid concentration by the method of SMITH et al. [15].

Results

Group I

a) Systemic circulation. In response to bleeding cardiac output decreased from 1.85 $\pm$ 0.1 to 0.94 $\pm$ 0.07 L/min. At 60 mm Hg arterial pressure, mannitol increased it to 1.44 $\pm$ 0.5 L/min. The effect was based on a significant (33 per cent) decrease of TPR, which in the first periods was 67 $\pm$ 6.5 and 61 $\pm$ $\pm$ 6.4 $\times 10^2$ dyne sec cm^{-5}; in the hypotensive ones, 58 $\pm$ 5.3; during mannitol infusion 39 $\pm$ 3.8 $\times 10^2$ dyne sec cm^{-5}. After retransfusion cardiac output

returned to the initial level (1.83 ± 0.17 L/min). TPR, however, remained low (51 ± 4.0), correspondingly blood pressure rose only to 100 ± 3.9 mm Hg, thus did not reach the initial mean of 133 ± 3.6 mm Hg. This was due, at least in part, to the fact that the viscosity of blood remained low. The venous haematocrit was namely 46 ± 1.2 at the onset of the experiment, 42 ± 1.5

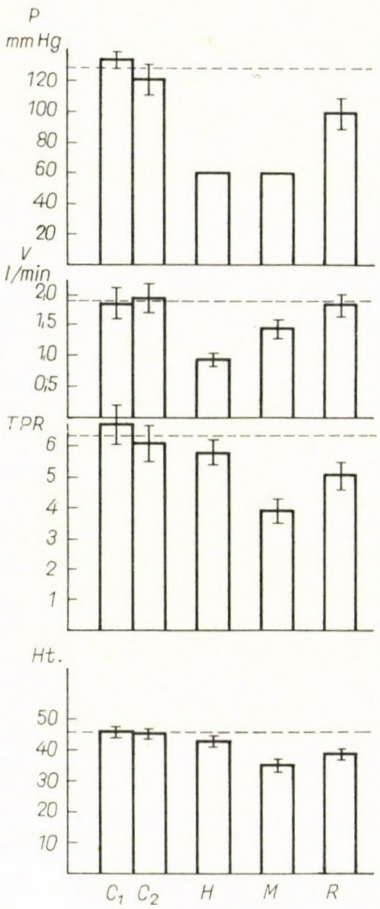

Fig. 1. Effect of hypertonic mannitol infusion at constant arterial pressure on blood pressure (P), cardiac output (V), systemic vascular resistance (TPR) and venous haematocrit (Ht). C_1 and C_2: control periods. H: hypotensive period. M: during mannitol infusion. R: period following retransfusion

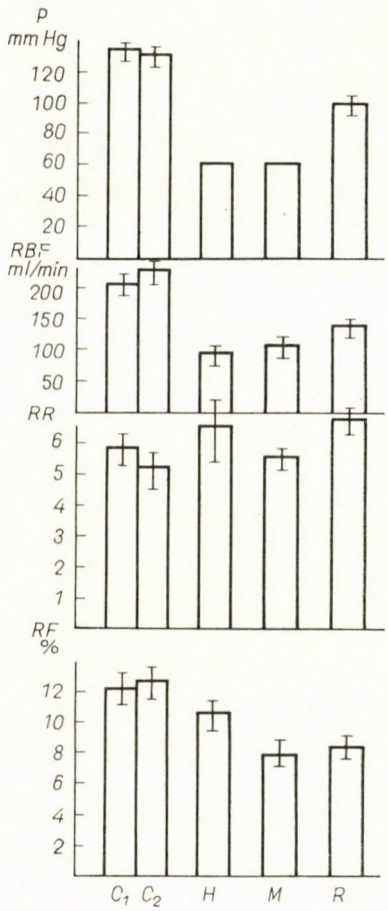

Fig. 2. Effect of hypertonic mannitol infusion on arterial pressure (P), left renal blood flow (RBF), renal resistance (RR) and the renal fraction of cardiac output (RF). Signs denoting periods as in *Fig. 1*

during bleeding, and 34 ± 1.6 in response to mannitol infusion, and did not rise above 37 ± 1.2 following retransfusion.

b) Renal circulation. The changes in RBF were similar to those of cardiac output: during hypotension RBF_{dir} dropped to half the initial value, from

203 ± 13.5 ml/min to 96 ± 9.2 ml/min. However, in response to mannitol infusion RBF increased only slightly, to 106 ± 12.5 ml/min. This means that the renal fraction of cardiac output (as examined in the left kidney) dropped from the pre-bleeding 12 per cent to 10.3, and continued to decrease in response to mannitol, to 7.7 per cent. At any rate, some decrease could be demonstrated

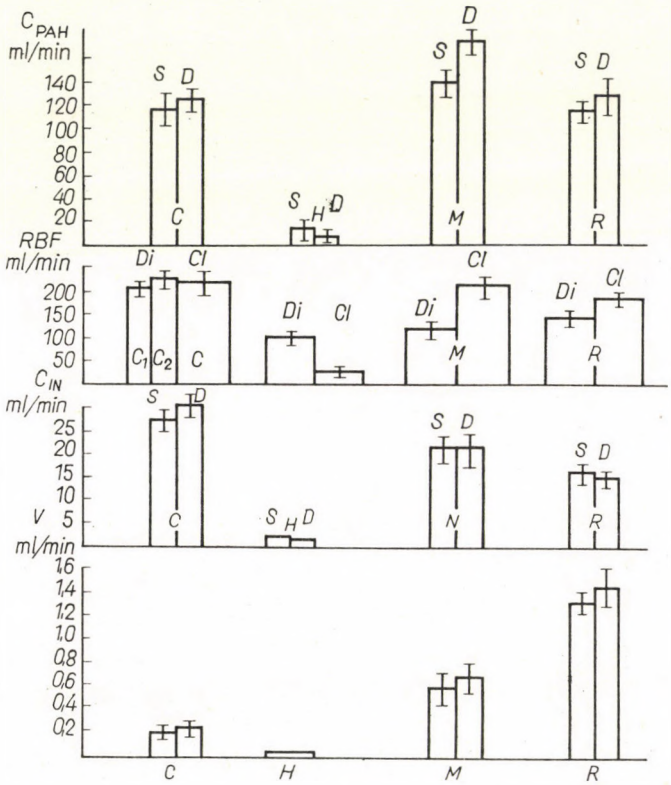

Fig. 3. Effect of hypertonic mannitol infusion at constant arterial pressure on C_{PAH} and C_{in} directly measured and calculated RBF, and on diuresis (V ml/min). S: left kidney. D: right kidney. Di: RBF measured directly. Cl: RBF calculated from C_{PAH} of left kidney. Other abbreviations and signs as in *Fig. 1*

also in renal vascular resistance, which was 58 ± 4.7×10³ dyne sec cm^{-5} at the beginning of the experiment, 64 ± 10.0 after bleeding, and 55 ± 2.7 after mannitol infusion. The mean decrease of 16.5 per cent was significant by the one sample "t" test ($p < 0.05$).

c) Renal function. Urine output was almost equal in the two kidneys; at the beginning of the experiment it was 0.21 ± 0.05 ml/min in the intact right kidney and 0.17 ± 0.03 ml/min in the left one. During bleeding diuresis stopped in most cases, or was reduced to inmeasurable levels. In spite of the fact that

blood pressure remained unchanged, mannitol infusion increased diuresis to 0.71 ± 0.11 and 0.59 ± 0.12 ml/min., respectively. After retransfusion diuresis increased to double that value (1.95 ± 0.20 and 1.34 ± 0.18 ml/min., respectively).

There was hardly any difference between the two kindeys in inulin clearance: 30 ± 2.5 ml and 27 ± 1.5 ml. During oliguria this decreased almost to zero (1.5 and 1.4 ml, respectively), in response to mannitol infusion it increased again to 21 ± 2.5 and 21 ± 2.8 ml., respectively. It is likely that during anuria some quantity of inulin had been accumulated and this was eliminated when diuresis started again. This is suggested namely by the observation that following retransfusion inulin clearance decreased to 15 ± 1.1 and 16 ± 1.9 ml/min, respectively.

The PAH clearances were closely similar in the preliminary periods: 126 ± 10.1 and 117 ± 13.1. During hypotension C_{PAH} dropped to almost zero. Mannitol infusion strongly increased C_{PAH}, to 175 ± 28.4 on the right side and 139 ± 11.2 ml/min on the left. Here, too, an accumulation of PAH during oliguria must have taken place in the kidney. Following retransfusion the C_{PAH} showed namely a decrease (128 ± 18.1 and 114 ± 8.3). E_{PAH} decreased during the experiment and even negative values were obtained in a few cases during oliguria or during mannitol infusion.

RBF calculated from C_{PAH} and venous haematocrit increased more markedly during mannitol infusion in the left kidney than did the RBF_{dir}. In the preliminary periods in the left kidney the RBF calculated from C_{PAH} and haematocrit was 215 ± 26 ml/min: exactly the same as the mean of direct measurements. Following mannitol infusion the value increased to 211 ± 21 ml, as compared with the directly measured 106 ± 12 ml. After retransfusion the calculated RBF decreased to 180 ± 16 ml, but this was still higher than the RBF_{dir} value.

Group II

a) Systemic circulation. Bleeding to 60 mm Hg arterial pressure reduced cardiac output from 1.75 ± 0.16 and 1.71 ± 0.16 L/min. to 0.89 ± 0.06. This was significantly increased by mannitol infusion, to 2.95 ± 0.31 L/min, whereas retransfusion had no influence on cardiac output (2.64 ± 0.31 L/min). Blood pressure increased significantly during mannitol infusion. While at the beginning of the experiment arterial pressure was 124 ± 3.8 and 119 ± 3.6 mm Hg, and after the blood loss it was reduced to 60 mm Hg, in response to 200 ml of hypertonic mannitol arterial pressure increased again to 89 ± 3.1 mm Hg. Retransfusion of the lost 240 ± 28 ml of blood increased blood pressure to 99 ± 5.9 mm Hg, i.e. arterial blood pressure was not restored to the initial level.

Mannitol significantly diminished TPR. This was 64 ± 6.5 and 63 ± ± 7.2×10^2 dyne sec cm^{-5} at the beginning of the experiment, the value during

hypotension was 59 ± 6.1, during mannitol infusion 27 ± 2.6 units, and 34 ± 4.6 after the retransfusion of blood. This was due, at least in part, to a reduction of blood viscosity. The hematocrit decreased from the pre-experi-

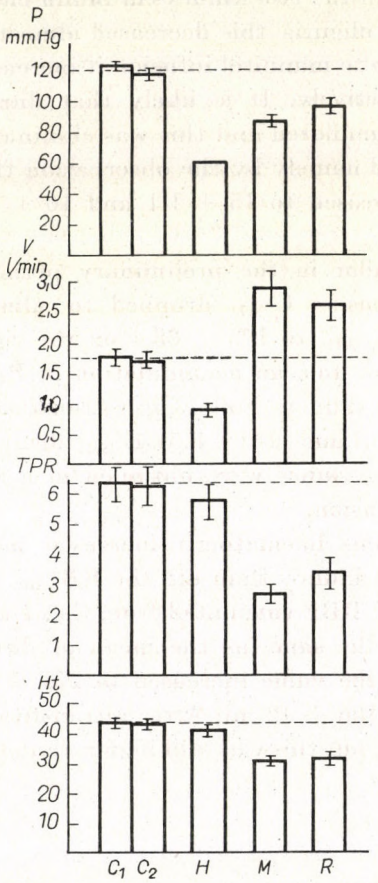

Fig. 4. Effect of hypertonic mannitol solution, during increasing circulating blood volume and arterial pressure, on blood pressure (P), cardiac output (V); total peripheral vascular resistance (TPR), and the haematocrit (Ht)

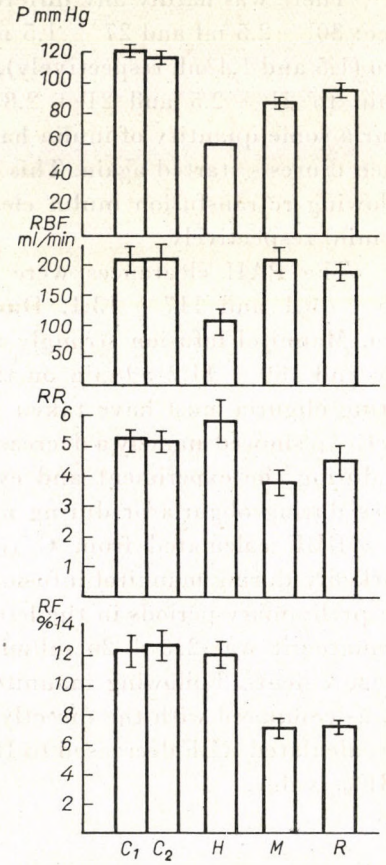

Fig. 5. Effect of hypertonic mannitol solution on renal haemodynamics during increasing circulating plasma volume and arterial pressure. Abbreviations and signs as in *Fig. 1* and *Fig. 2*

mental 43 ± 1.6 to 30 ± 1.7 during infusion, and retransfusion hardly influenced the value (32 ± 1.7).

b) Renal circulation. In the preliminary periods RBF_{dir} was 213 ± 23.1. During hypotension this decreased to 107 ± 20.3 ml/min. Mannitol infusion increased it to 209 ± 23.7. After retransfusion RBF decreased (not significantly) to 187 ± 11.7.

Renal vascular resistance was 52 $\pm$ 5.7 $\times$ 103 dyne sec cm^{-5}. Following blood loss it increased insignificantly to 58 $\pm$ 7.1. Mannitol infusion was followed by a very highly significant decrease, to 38 $\pm$ 4.2 units ($p < 0.01$). Retransfusion increased the vascular resistance to 46 $\pm$ 5.2.

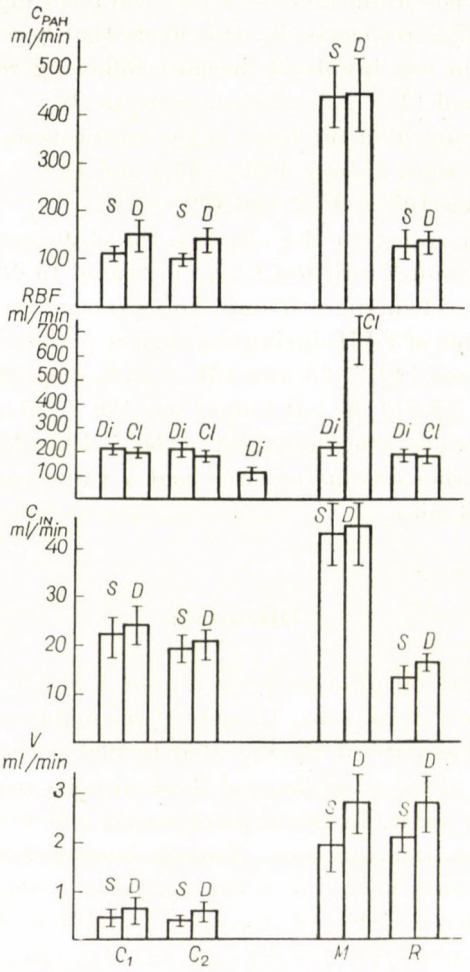

Fig. 6. Effect of hypertonic mannitol solution on PAH and inulin clearances, on diuresis, as well as on the directly measured and calculated renal blood flow, during increasing circulating plasma volume and arterial pressure. Abbreviations and signs as in *Fig. 1* and *Fig. 3*

c) Renal function. During the two preliminary periods diuresis in the left kidney was 0.38 $\pm$ 0.12 and 0.34 $\pm$ 0.07, in the right kidney 0.55 $\pm$ 0.24 and 0.52 $\pm$ 0.22 ml/min. During hypotension the animals were practically anuric, none of them showed diuresis reaching 0.05 ml/min. In response to mannitol

diuresis increased highly significantly, to 2.71 ± 0.64 on the right and to 1.83 ± 0.51 on the left side. Retransfusion had no significant influence (2.72 ± ± 0.57 and 2.01 ± 0.27 ml/min, respectively).

The initial inulin clearance values were 24 ± 3.7 and 20 ± 3.3 for the right kidney and 21 ± 4.0 and 19 ± 3.1 ml for the left. Mannitol infusion administered during hypotension increased the clearances highly significantly, to 44 ± 8.8 and 43 ± 12.2, respectively. It is likely that here, too, an elimination of accumulated inulin was involved, because following retransfusion C_{in} was as low as 16 ± 2.1 and 13 ± 2.5 ml/min, respectively.

C_{PAH}, as determined on the basis of the arterio-venous difference, was at the beginning in the right kidney 149 ± 33.9 and 139 ± 33.6 ml/min, and in the cannulated left one 107 ± 14.2 and 100 ± 5.8.

In hypotension, owing to the oliguria, the clearances could not be estimated. Following mannitol infusion C_{PAH} increased to 466 ± 85 in the right kidney and to 441 ± 71 in the left one. This extreme increase was due here, too, to an accumulation of PAH during the oliguric period. The clearance values after retransfusion were 139 ± 23 and 131 ± 26.8, respectively.

The calculated RBF of the left kidney was 190 ± 25 and 181 ± 11 ml/min in the preliminary periods. This increased to 666 ± 105 ml/min during mannitol infusion, while following retransfusion it had a value comparable to that of RBF_{dir} (178 ± 39 ml/min).

Discussion

Hypertonic mannitol solution proved to be a highly potent diuretic also under our experimental conditions. In spite of the maintenance of hypotension at the same level and in spite of the fact that in that period the volume of lost blood exceeded that of the administered fluid, diuresis rose to three times the normotensive control value. In the experiments in which no further blood loss occurred during mannitol infusion, diuresis increased more than fivefold, although RBF was much lower than the control in group I and near to it in group II. The correlation between diuresis and GFR is difficult to evaluate, because, in contrast to the view of PETERS and BRUNNER [11], we think that during anuria inulin may accumulate in the kidney and this is eliminated when in response to mannitol diuresis starts again. This hypothesis is supported by the fact that in the next clearance period C_{in} decreased, in spite of the fact that the total volume of blood had been retransfused.

Mannitol increased RBF only moderately, by 11 per cent, when arterial pressure was maintained at a constant level. Correspondingly, the decrease of renal vascular resistance was slight; at any rate, the decrease was less than the 33 per cent decrease demonstrated in TPR.

In the experiments in which the further loss of blood was prevented during mannitol diuresis, RBF became almost normal, although arterial pressure remained under its value before bleeding. Correspondingly, renal vascular resistance decreased by 34 per cent. However, TPR decreased even more markedly. Otherwise, the mannitol significantly increased cardiac output in both types of experiment. These observations are at variance with the view that hypertonic mannitol would exert a specific renal effect [6, 8].

Mannitol was, however, shown to diminish vascular resistance in the isolated kidney [5, 4], therefore the effect cannot be ascribed simply to an increase of plasma volume and cardiac output. This direct renal effect may only in part be due to a decrease of blood viscosity, because RBF increased even when pressure was kept constant and the haematocrit values were unchanged [4].

As mentioned, in our experiments mannitol significantly increased cardiac output and diminished TPR. However, even when it is brought about by homologous plasma infusion, a normovolaemic or hypervolaemic decrease of blood viscosity will significantly increase cardiac output and diminish TPR [9]. Accordingly, the systemic haemodynamical effects of mannitol might be explained by a decrease of blood viscosity. In our experiments the systemic haemodynamic effect was more marked when haemodilution was not followed by a further loss of blood, i.e. when together with haemodilution the circulating blood volume increased significantly. This is analogous to the observation that in response to a decrease of haematocrit brought about by plasma infusion, the cardiac output increases much more significantly when the decrease of blood viscosity is associated with hypervolaemia.

In our experiments the increase of cardiac output and the decrease of TPR exceeded in measure the changes in renal haemodynamics. To this may be ascribed the finding that in spite of the increase of RBF, the renal fraction of cardiac output significantly decreased in both types of experiment: in response to mannitol in the left kidney the renal fraction of cardiac output decreased from 12 per cent to 7.7 and 7 per cent, respectively.

Thus, the effect of mannitol on systemic circulation was essentially the same as its renal haemodynamic effect, only the former was more marked. The increase in cardiac output may be explained by the increase of circulating plasma or blood volume. It is highly probable that the volume increased in group I, too, in which arterial pressure was kept low by further bleeding. That this was so, is suggested by the decrease of vascular resistance when arterial pressure was constant, from which one may conclude to an increase in the capacity of the vascular bed, and consequently also to that of the blood volume filling it. In a few cases we measured by means of radioactive human serum albumin the circulating plasma volume before and after mannitol infusion. It has been found that although the animals have lost blood in

excess of the volume of infused fluid, the volume of plasma still increased. It has also been suggested that mannitol might act as a vasodilator. The evidence obtained indicated that the decrease of renal resistance cannot be based on a decrease of blood viscosity alone [6]. In our case, too, the more than 60 per cent decrease in TPR can be ascribed only in part to the 30 per cent decrease of the haematocrit (from 43 to 32), and a direct vasodilator effect of hypertonic mannitol must be assumed.

The mechanism of the systemic vasodilator effect is not clear; the pertaining data tend to indicate that the effect is not neurogenic [5].

As far as practice is concerned, the systemic haemodynamic effect of mannitol appears to be advantageous in posttraumatic and haemorrhagic shock. On the one hand, by its osmotic action it will increase circulating blood volume and thus cardiac output, and, on the other hand, it causes peripheral vasodilatation, just like adrenergic blockade, e.g. phenoxybenzamine, does, and enhances blood flow in certain organs. It appears to be a disadvantage that as a result of its direct renal effect, mannitol causes a considerable loss of water and electrolytes [16, 13, 17]. This effect, however, is of no practical importance when the patient's life is in acute danger, and the losses of water and electrolytes may easily be corrected [2]. Thus, apart from its use in the treatment of anuria and the prevention of renal lesions, hypertonic mannitol is therefore a useful agent in such conditions.

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THE ERYSIPELAS PROBLEM

II. THE PROPERTIES AND AETIOLOGICAL ROLE OF STREPTOCOCCUS STRAINS ISOLATED FROM ERYSIPELAS AND OTHER DERMAL DISEASES

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From 115 patients with erysipelas and 214 patients suffering from eczema or pyoderma, 66 beta-haemolytic streptococcus strains have been isolated. Of the strains 25 were group A, 22 group C and 19 group G. The A group strains belonged to 12 different types. Of the isolated 66 strains 64 were tested in mice. Most of the strains were avirulent and a few were moderately virulent. Since the strains which proved to be avirulent in the mouse caused serious disease in man, and as the behaviour of the delayed-type hypersensitivity reaction, and the absence of true immunity suggested an immune defect, it may be assumed that the human pathogenicity of the strains avirulent in the mouse may be attributed to a defect of the defence system. The weakness of the antigen and the diminished ability of the organism to produce specific antibodies may account for the frequent recurrence of erysipelas.

In the first paper of this series [1] the results of delayed-type skin tests involving the use of various streptococcal antigens allowed the conclusion that in erysipelas a certain kind of immune weakness exists as far as delayed-type reactivity is concerned. This conclusion is in harmony with the long-known fact that erysipelas does not leave behind immunity, as well as with the finding that leucocytes, including the small lymphocytes responsible for delayed-type hypersensitivity, are almost entirely absent in the areas of inflammation [2]. In order to further elucidate the pathomechanism of erysipelas, it has been deemed necessary to study the distribution of the types and virulence of the pathogenic streptococcal strains, the more so as apparently Bartels and Riskaer in 1944 were the last to report on the isolation of streptococci from cases of erysipelas [3] and because the strains of streptococci isolated from erysipelas do not seem to have ever been tested for virulence.

Materials and methods

Material tested. From affected skin areas, samples of tissue-fluid were taken under aseptic conditions in 115 cases of erysipelas and 214 cases of microbial eczema or pyoderma, to isolate bacteria. At the same time, blood samples were obtained for serological testing.

Cultivation. The tissue fluid or discharge was injected directly into broth (Szita—Bartha's medium, [4]) and the beta-haemolytic streptococci were isolated on bovine blood containing agar plates.

Determination of groups and types. The streptococcus strains were grouped by use of the Lancefield extract, repeating the determination in doubtful cases with the Fuller extract.

Typing on the basis of the T-antigen was carried out by the agglutination technique of *Griffith* and on grounds of the M-antigen by the precipitation test of *Lancefield* as described previously [5].

Test for virulence. From the 6-hour culture of the strain inoculated into KALBAK's medium [6] tenfold dilutions were made from 1 in 10 to 1 in 10 million, and white mice, weighing 18 to 20 g were inoculated with 1 ml doses intraperitoneally, using 4 mice for every dilution, As determined after 5 days of observation, a strain was considered virulent when it killed at least 2 mice at a dilution of 1 in 1000 or higher. The germ counts of the 6 hour cultures were between 10^7—10^8/ml.

Antibody titration. The antistreptolysin-O (ASO) and the antistreptokinase (ASK) titres were determined according to BÖSZÖRMÉNYI [7, 8]. In normal adults both titres average 125 units. In Hungary the highest limit of normal is estimated 250 U [9, 10].

Results

The bacterological results for 329 patients are presented in *Table I.* As this paper is intended to deal solely with the role of streptococci, the results concerning them will only be discussed. From the two groups of patients a total of 66 strains of haemolytic streptococci could be isolated. Their groups and types are shown in *Table II.*

Table I

Cultivation results

Diagnosis	Isolated microorganism	Number of cases	Per cent
Erysipelas	β-haemolytic streptococcus	21	18,3
	Facultative pathogens	51	44.3
	Non-pathogenic bacteria	31	27.0
	None (sterile culture)	12	10.4
	Total	115	100.0
Microbial eczema and pyoderma	β-haemolytic streptococcus	45	21.0
	Facultative pathogens	126	58.9
	Non-pathogenic bacteria	33	15.4
	None (sterile culture)	10	4.6
	Total	214	100.0

Facultative pathogens: staphylococci, non-haemolytic streptococci, B. proteus, Klebsiellae, Pseudomonas.

From all three groups of diseases, C and G-group streptococci could be isolated in relatively large numbers. The incidence of the latter and of A-group streptococci is shown separately in *Table III.* The 25 A-group strains isolated belonged to 12 different types, thus, in agreement with other authors, we found no type to preponderate in any disease. This of course means that the examined diseases were not related to special serotypes.

Table II

Serological distribution of beta-haemolytic streptococci isolated from various skin diseases

Groups	Erysipelas	Microbial eczema	Pyoderma	Total	Per cent
A	8	14	3	25	37.9
C	8	9	5	22	33.3
G	5	9	5	19	28.8
Total	21	32	13	66	100

Types in Group A	Number of single types			Total
3	1			1
3, 13	1	2	1	4
5		1		1
5, 11, 12		3		3
8		1		1
8, 25	2	3		5
9			1	1
12	1			1
15			1	1
24	1	1		2
25		2		2
Untypeable	2	1		3
Total	8	14	3	25

Table III

Relative incidence of A and C + G-group streptococci in the three groups of skin diseases

	Erysipelas per cent	Microbial eczema per cent	Pyoderma per cent
Group A	38	44	24
Group C + G	62	56	76
	100	100	100

Immediately after isolation, 64 strains out of the total of 66 were tested for virulence in mice, with the aim to determine the protective antibody titres of the patients' sera against the strains causing disease in them. This attempt

Table IV

Virulence tests

Number of mice killed in groups of 4 mice each by 10^{-1} to 10^{-6} dilutions of different strains of streptococcus

Diagnosis	Str. Group A						Str. Group C				Str. Group G			
	Dilution													
	No*	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5} 10^{-6}	No.	10^{-1}	10^{-2}	10^{-3}	No.	10^{-1}	10^{-2}	10^{-3}
Erysipelas	1	2					9	1			17	1		
	2	4	3				10				18	2		
	3	4	1				11	3			19			
	4	2					12	1			20	3	1	
	5	3	2				13				21			
	6	1	1				14							
	7	not tested					15	2						
	8						16							
Microbial eczema	22						36	2			45			
	23	1					37	2	1		46	2	2	
	24	1					38	3	2		47	3	2	
	25	2	1				39	4	2		48	1		
	26						40	1			49	1		
	27						41	2	1		50	4	3	2
	28	1					42	1			51	2	1	
	29						43				52	2	1	
	30	2					44	1			53	3		
	31													
	32	1												
	33													
	34													
	35													
Pyoderma	54	4	4	1			57	2			62	1		
	55	1					58	2			63	1		
	56	1					59	2	2		64			
							60	4	2		65	1	1	
							61	1			66	4	3	2
Scarlet fever	67	4	4	3	3	2 1								
	68	2	2											
	69	4	3	3	2									
	70													
	71	4	4	3	2	2 2								

Diagnosis	Str. Group A					
	Dilution					
	No.*	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Tonsillitis	72					
	73	3	1			
	74	4	3			
	75	4	4	1		
Otitis	76	4	4	3	2	
	77	4	3	2	1	
Meningitis	78					
Postoperative suppuration	79	1	1			

* No. of strains

failed, since the virulence of the first isolated strains was so low that it was impossible to set up a passive protection test against them. In view of these results increased attention was focussed on the virulence of the isolated strains, but the later results were similar to the earlier ones and there was no strain which would have been lethal to white mice in higher dilution as 1 in 10,000.

Table V

Antistreptolysin-O (ASO) and antistreptokinase (ASK) titres in streptococcus-positive skin diseases

Titre	Number of cases			
	Erysipelas		Microbial eczema and pyoderma	
	ASO	ASK	ASO	ASK
15—25 U (units)		2		2
30—50 U		1		1
60—100 U	1	2		2
120—200 U	7	3	2	5
240—400 U	6	7	12	3
480—800 U	3	1	6	4
960—1600 U	3	1	4	1
1920—3200 U			1	1
3840—6400 U				2
Total	20	17	25	21
Mean titre, units	340	175	420	262

To control the technique of isolation and that of virulence tests, 13 A-group streptococcus strains were tested for virulence shortly after isolation from patients with scarlet fever, tonsillitis and otitis. Results are presented in *Table IV*. As visible, 44 strains of the 64 isolated from skin diseases were absolutely avirulent and the remaining 20 were lethal for white mice at a dilution of 1 in 100. At the same time, of the 13 control strains only 3 were avirulent, and 5 were lethal even at a dilution of 1 in 10,000.

In order to determine whether the streptococci isolated from skin diseases represented an antigenic stimulus, at all for the organism, in 45 out of the 66 streptococcus-positive cases the sera were tested for ASO titre and in 38, for ASK titre. Results and calculated mean titres are shown in *Table V*. Although in the majority of cases the titres of both antibodies were higher than 250 U, they exceeded this upper normal limit only slightly. In the erysipelas group the mean titres for both antibodies were lower than in the streptococcus-positive patients with eczema or pyoderma.

Discussion

Group C and G strains were frequent among the isolated streptococcal strains (33 and 29 per cent, respectively). Other authors have not observed similar incidences, and during the past 20 years nobody seems to have studied strains isolated from erysipelas. According to the statistics published in this country [5] and abroad [11, 12, 13] 70 to 95 per cent of the streptococci isolated from scarlet fever, tonsillitis and septicaemia were A-group and only 3 to 15 per cent were C and G-group strains. According to the same statistics C and G-group streptococci are more frequent in healthy carriers than in patients with acute illnesses. The comparatively low pathogenicity of group C and G strains has also been known for long.

The high incidence of C and G strains in our material raises the question whether they were actually pathogenic or were isolated only by chance, as opposed to the A-group strains, which are certainly pathogenic and whose isolation is not a matter of chance. It was remarkable that the isolated group-A strains should be just as little pathogenic for mice as the C and G-group strains. The question thus arose whether the strains avirulent in the mouse are capable of producing disease in man. According to the prevalent view, for which most of the credit goes to LANCEFIELD [14], the virulence factor and the protective antigen of the streptococci is identical with the type-specific M protein.

True immunity is provided for exclusively by the type-specific anti-M antibodies. If an individual is infected by a virulent streptococcal strain, after recovery he will have type-specific immunity, which may persist for several decades [15].

Thus, according to the above theory the recurrences of streptococcal diseases would always have to be caused by different types. But in certain individuals erysipelas is known to have recurred more than 50 times, i.e. more than the total number of types of Group A streptococci. This obviously means that in such cases the same type had to be the pathogenic agent at least twice. BARTELS and RISKAER [3] reported two, HOLMES and WILLIAMS [16] nine, KÖHLER and HEINRICH [17] four patients in whom after some months interval the same type of streptococcal A-group had caused for the second time a new acute disease.

Therefore, clinical immunity does not necessarily develop even to the type of streptococcus which has already caused manifest disease. At least two hypotheses may be put forward to explain this: either the pathogenic strain was not antigenic enough, and/or the immune apparatus of the organism reacted weakly.

Thus, it seems reasonable to claim that streptococcal strains avirulent in the mouse are capable of causing erysipelas or other disease in man, as it has been concluded from clinical observations, in the first place. There is a marked discrepancy between the results of virulence tests and the clinical symptoms of erysipelas, inasmuch as the strains isolated from erysipelas and nonvirulent for the mouse are highly pathogenic for man; they may produce serious disease with high fever, malaise, intensive hyperaemic-oedematous inflammation. The pathogenicity of avirulent strains in animals is confirmed by the recent investigations of CAYEUX et al. [18], who could induce severe cardiac and articular changes in mice if the infection with streptococcus strains avirulent for mice was coupled with the inhibition of leucocytes by antileucocytic serum. The analogy between Cayeux's avirulent laboratory strains and the strains isolated by us is obvious, except that the said authors have damaged the defence system of mice, while in erysipelas there may be a defect in the defence system of the organism due to some as yet unclear cause.

The latter possibility is supported by the fact that in our erysipelas patients the ASO and ASK antibody titres were lower than in the patients with streptococcus-positive eczema and pyoderma, and much lower than for example in rheumatic fever, chorea and other streptococcal diseases [19].

A further sign indicative of the weak reactivity of the immune apparatus was that the delayed-type allergic skin reactions of the patients with recurrent erysipelas were less frequent positive than in the controls [1]. Still another sign of the poor reactivity of the immune apparatus was the fact that in the inflamed areas of erysipelas the number of leucocytes (including the small lymphocytes i.e. immunocytes) was remarkably low [20, 2].

Just this immunological weakness may be responsible for the fact that under certain predisposing circumstances avirulent or less virulent streptococci, too, may cause disease in man, especially when they encounter conditions

favourable for their growth and multiplication (in erysipelas mainly in the lymphatics). In this case they manifest themselves with grave and peculiar symptoms. The streptococcal strains avirulent for mice, i.e. those producing no M-antigen, are namely not deficient in any other respect, since their ability to multiply and to produce toxic extracellular substances is independent of the formation of M-antigen, and it has been shown in rabbit experiments that certain streptococcus fractions not containing M-antigen may give rise to strong delayed-type sensitization [21]. These toxic factors may by themselves evoke serious pathological symptoms even in the absence of M-antigen, when the immune activity of the organism is weak.

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ÜBER DIE BEDEUTUNG DER BAKTERIÄMIE UND TOXINÄMIE IM ZUSTANDEKOMMEN DER OLIGO-ANURIE

Von

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Im Verlauf von 6 1/2 Jahren wurden 87 Oligo-Anurien bakteriämischen-toxinämischen Ursprungs beobachtet. Die Gesamtmortalität betrug 49,4% (43 Fälle). Symmetrische Nierenrindennekrose war in 8 Fällen (Mortalität 100%), Schockniere in 56 Fällen (Mortalität 32,1%), akute Aufflackerung einer chronischen Nierenkrankheit in 23 Fällen (Mortalität 74%) zu verzeichnen. Die auslösenden Ursachen der Nierenrindennekrose bzw. der Schockniere waren in erster Reihe septischer Abort, die der chronischen Nierenkrankheit (hauptsächlich Pyelonephritis chronica) dagegen Cholezystitis, Cholangitis und Cholangiolitis.

Tierexperimentelle Untersuchungen und klinische Beobachtungen sprechen dafür, daß auf Wirkung von Bakteriämie und Toxinämie verschiedene Nierenveränderungen zustandekommen können. In vorliegender Arbeit teilten wir die Patienten unserer Kunstnierenstation, bei denen die Entstehungsursache der Oligo-Anurie Bakteriämie bzw. Toxinämie war, den Nierenveränderungen entsprechend in 3 Gruppen ein.

Krankenmaterial

1. *Symmetrische Nierenrindennekrose.* Wie darauf sämtliche sich mit Nierenrindennekrose befassenden Mitteilungen und Monographien hinweisen, kann in der Entstehung dieses Nierenprozesses unter anderen auch die Infektion eine Rolle spielen.

An den Kunstnierenstationen ist die nach septischem Abort auftretende Rindennekrose wohlbekannt. Bei Kindern konnten McKAY und WAHLE [8], ESKELAND und SKOGRAND [5], ZUELZER und Mitarb. [13] sowie andere Verfasser einen Gram-negativen Krankheitserreger nachweisen; BOUISSON und Mitarb. [4] sowie NAKAI und MORGARETTEN [9] führten mit *E. coli*-Toxin experimentelle fleckige oder Totalnekrose herbei. In der von JUHEL-RÉNOY [7] 1886 publizierten Mitteilung, in der die beiderseitige Nierenrindennekrose das erstemal beschrieben wurde, war die Ursache der Krankheit eine Streptokokken-Infektion. In den Fällen von WEAVER und HAAM [12] sowie anderer

Verfasser entwickelte sich die Nekrose infolge Streptokokken-Tonsillitis. Die symmetrische Nierenrindennekrose kann bei Versuchstieren auch mit Streptokokken-Lysat erzeugt werden. GLAHN und WELD [6], NAVASQUEZ [10] und andere Verfasser beobachteten durch Staphylokokken-Toxin herbeigeführte Rindennekrose. Pneumokokkenbedingte Nekrose konnten BLACKMAN und Mitarb. [3] bei Kindern nach Lungenentzündung und BELL [2] nach Pneumokokken-Peritonitis wahrnehmen. Unser Ziel ist nicht die eingehende Erörterung der ausgedehnten Literatur, da dies den Rahmen dieser Arbeit überschreiten würde, wir wollen lediglich diejenigen Mitteilungen hervorheben, in denen über Krankheitserreger berichtet wird, die auch in der menschlichen Pathologie eine Rolle spielen können und in einigen unserer Fälle für die Entwicklung der Nierenrindennekrose verantwortlich waren.

In unserem Material kamen 9 Fälle mit Nierenrindennekrose vor: In 8 dieser Fälle (1,6% des Gesamtmaterials) lag als auslösende Ursache Sepsis vor. Der septische Zustand entwickelte sich in 5 Fällen infolge von septischem Abort, in 2 infolge Cholezystitis und in 1 Fall infolge Pneumonie (Tab. I).

Tabelle I

Symmetrische Nierenrindennekrose

Nr.	Name	Grundkrankheit
1.	Frau L. P.	Septischer Abort (fleckige Nierenrindennekrose)
2.	Frau J. Sz.	Septischer Abort
3.	Frau J. B.	Septischer Abort
4.	Frau P. J.	Septischer Abort
5.	Frau J. H.	Septischer Abort
6.	Frau Gy. V.	Cholecystitis perforativa, Peritonitis
7.	L. V.	Cholecystitis, Cholangiolitis
8.	Frau F. V.	Pneumonie

Falldarstellung. Frau L. P. Bei der 21jährigen Patientin wurde die Nierenrindennekrose mittels Biopsie in vivo diagnostiziert. 30. IX. 1961 wurde eine etwa 3—4monatige Schwangerschaft unterbrochen, und es traten Blutung, Schüttelfrost, Sepsis und Gelbsucht auf. Patientin stellte im Zusammenhang mit der Schwangerschaftsunterbrechung die Kriminalität entschieden in Abrede. Die 8 Tage hindurch andauernde massive Anurie konnte durch konservative Behandlung und 2malige Hämodialyse behoben werden, und die Nierenfunktion setzte sich allmählich in Gang, die tägliche Harnmenge betrug aber selbst nach 20 Tagen lediglich 100—200 ml. Wegen des Verdachtes einer Nierenrindennekrose wurde eine Nierenbiopsie durchgeführt. Der größte Teil des histologischen Präparats zeigte lediglich das Schattenbild der Nierenstruktur, Kernfärbung war nicht zu beobachten; an einem kleineren Teil des histologischen Schnittes war in der Nierensubstanz Kernfärbung ersichtlich. Auf diesem Gebiet traten auch einige hyperämische Glomerulen in Erscheinung. Von diesem Zeitpunkt an begann ein langsamer Anstieg der Diurese, die Menge des 24-Stundenharns blieb aber unter 1700 ml (spez. Gewicht: 1006—1008). Der Reststickstoffwert schwankte um 110 mg%, in dieser Zeit war Patientin nicht mehr bettlägerig. 9. X. eklamptische Anfallserie, Tod. Nach den Zimmer-

genossinnen überschritt Patientin die diätetischen Vorschriften, indem sie die Speisen heimlich salzte und würzte.

Nach septischem Abort kann somit auch eine Nierenrindennekrose fleckigen Typs auftreten. Anhand des histologischen Bildes steht es aber fest, daß das Leben der Patientin, selbst wenn der eklamptische Anfall nicht dazwischen gekommen wäre, nur für kurze Zeit zu verlängern gewesen wäre.

2. *Schockniere.* An dieser Stelle wollen wir selbstverständlich nur über jene Fälle berichten, in denen sich die Oligo-Anurie, d. h. die akute Niereninsuffizienz auf Grund eines bakteriellen bzw. toxinämischen Schocks entwickelte. Anschließend sei betont, daß der Schock nicht unbedingt zur akuten Niereninsuffizienz führt. Im Zusammenhang mit Schock kommt es zwar häufig zur Nierenschädigung (»Niere im Schock«), das Maß derselben ist jedoch nicht immer derartig bedeutend, daß sich Oligo-Anurie entwickeln würde.

Tabelle II

Schockniere

Nr.	Name	Diagnose	Lebt	Gestorben
		Septischer Abort (50 Fälle)	36	14
1.	N. D.	Eiterung nach Herniotomie		+
2.	Á. F.	Pankreatitis-Peritonitis		+
3.	Frau I. G.	Glutealabszeß		+
4.	D. N.	Pararektale Eiterung		+
5.	I. L.	Ventrofixation, Peritonitis	+	
6.	I. M.	Septische Cholangitis, Cholangiolitis	+	
		Insgesamt	38	18

In 56 unserer Fälle war unmittelbar vor der oligo-anurischen Phase Schock oder hochgradige Blutdruckverminderung nachzuweisen (Tab. II). In 50 dieser Fälle war die auslösende Ursache ein septischer Abort. In 6 Fällen waren für die Bakteriämie-Toxinämie folgende pathologische Prozesse verantwortlich: Eiterung nach Herniotomie, Pankreatitis-Peritonitis, glutealer Abszeß, pararektale Eiterung, nach Ventrofixation aufgetretene Peritonitis, nach Gallenoperation aufgetretene Cholangitis, Cholangiolitis. Mortalität: in der ersten Gruppe (septischer Abort) 28% (14 Fälle), in der zweiten Gruppe 4 der 6 Kranke. Gesamtmortalität 32,1%.

3. *Akute Aufflackerung chronischer Nierenkrankheiten.* In einer vorangehenden Mitteilung (11) haben wir darauf hingewiesen, daß anläßlich der akuten Aufflackerung subakuter und chronischer Nierenkrankheiten (subakute und chronische Glomerulonephritis, chronische Pyelonephritis, Amyloidose, Nephrosklerose, Periarteriitis nodosa) Oligo-Anurie zustandekommen kann.

Tabelle III

Akute Aufflackerung chonischer Nierenkrankheiten

Nr.	Name	Grundkrankheit	Die die Aufflackerung auslösende Erkrankung	Lebt	Gestorben
1.	L. Sz.	Chronische Pyelonephritis	Cholezystitis gangraenosa		+
2.	Frau M. S.	Chronische Pyelonephritis	Cholezystitis, Cholangitis, Cholangiolitis acuta	+	
3.	Frau M. K.	Chronische Pyelonephritis	Cholezystitis, Cholangitis, Cholangiolitis acuta		+
4.	Frau F. S.	Chronische Pyelonephritis	Cholezystitis, Cholangitis, Cholangiolitis acuta		+
5.	Frau M. K. M.	Chronische Pyelonephritis	Cholezystitis, Cholangitis, Cholangiolitis acuta	+	
6.	M. T.	Chronische Pyelonephritis	Cholezystitis, Cholangitis, Cholangiolitis acuta	+	
7.	Frau J. B.	Chronische Pyelonephritis	Cholezystitis, Cholangitis, Cholangiolitis acuta	+	
8.	Frau I. V.	Chronische Pyelonephritis	Cholezystitis, Cholangitis, Cholangiolitis acuta		+
9.	Frau S. M.	Chronische Pyelonephritis	Eitrige Nephritis		+
10.	J. H.	Chronische Pyelonephritis	Eitrige Nephritis		+
11.	Frau Gy. U.	Chronische Pyelonephritis	Pyelonephritis acuta	+	
12.	Frau J. T.	Chronische Pyelonephritis	Pyonephros l. d. Pyelonephritis acuta		+
13.	Frau J. G.	Chronische Pyelonephritis	Glutealabszeß		+
14.	G. K.	Chronische Pyelonephritis	Glutealabszeß		+
15.	P. I.	Chronische Pyelonephritis	Glutealabszeß (Zuckerkrankheit)		+

Nr.	Name	Grundkrankheit	Die die Aufflackerung auslösende Erkrankung	Lebt	Gestorben
16.	P. Sz.	Chronische Pyelonephritis	Pankreas-Fettnekrose		+
17.	I. Sz.	Chronische Pyelonephritis	Pankreas-Fettnekrose		+
18.	Frau F. T.	Periarteriitis nodosa	Appendicitis gangraenosa, Peritonitis		+
19.	Frau I. K.	Chronische Pyelonephritis	Gallenblasenperforation, Peritonitis	+	
20.	Frau I. K.	Chronische Pyelonephritis	Bronchopneumonie		+
21.	Frau K. O.	Chronische Glomerulonephritis	Extrauterine Schwangerschaft-Operation		+
22.	B. J.	Amyloidose	Osteomyelitis		+
23.	K. V.	Chronische Glomerulonephritis	Enzephalitis		+
			Insgesamt	6	17

Die Ursache der akuten Exazerbation ist zumeist eine fiebrige Erkrankung. In vorliegender Arbeit befassen wir uns nur mit jenen Fällen, in denen für die akute Aufflackerung ein ohne Schock verlaufender septischer Zustand der Grund war (Tab. III).

Sepsisbedingte Aufflackerung einer chronischen Nierenkrankheit führte in 23 Fällen (4,62% des Gesamtmaterials) zu Oligo-Anurie. Die Ursachen der Sepsis waren wie folgt: Cholezystitis, Cholangitis, Cholangiolitis (8 Fälle), Fettnekrose (2 Fälle), Operation wegen extrauteriner Schwangerschaft (1 Fall), gangränöse Cholezystitis + Peritonitis (1 Fall), gangränöse Blinddarmentzündung + Bauchfellentzündung (1 Fall), Pyonephros (1 Fall), Enzephalitis (1 Fall), Pyelonephritis (1 Fall), Bronchopneumonie (1 Fall), Osteomyelitis (1 Fall).

Die Sepsis verlief in der Mehrzahl der Fälle an anderen Abteilungen, von wo dann die Patienten zu uns übergeliefert wurden. Die Krankheitserreger konnten wir nur in 2 Fällen (Fall 15: Staphylococcus haemolyticus und Fall 7: E. coli) identifizieren. Die Ursache der zahlreichen negativen Ergebnisse liegt darin, daß die Patienten vor der Aufnahme bereits mehrere Tage hindurch eine antibiotische Behandlung erhielten. Die Mortalität liegt recht hoch: 74% (die Gesamtmortalität der Kunstnierenabteilung macht 41% aus).

Histologisch waren in der Niere außer der Grundkrankheit zellreiche Glomerulen (Glomerulitis) sowie Infiltration und Ödem des Interstitiums festzustellen.

Die Grundkrankheit war in der Mehrzahl der Fälle chronische Pyelonephritis, in 2 Fällen chronische Glomerulonephritis und in je 1 Fall Amyloidose bzw. Periarteriitis nodosa.

Besprechung

An unserer Kunstnierenabteilung wurden zwischen 1. II. 1960—1. IX. 1966 497 Patienten behandelt; die Gesamtmortalität betrug 41%. In 87 Fällen (17,5% des gesamten anurischen Materials) handelte es sich um eine Oligo-Anurie bakteriämischen-toxinämischen Ursprungs. Die Mortalität dieser Gruppe — 49,4% — liegt höher als die des Gesamtmaterials. Anhand der Aufschlüsselung der bezüglichen Angaben läßt sich feststellen, daß die Prognose bei symmetrischer Nierenrindennekrose — verständlicherweise — außerordentlich schlecht ist; bei der akuten Aufflackerung chronischer Nierenkrankheiten sind die Aussichten ebenfalls schlecht, in Fällen, in denen lediglich eine Schockniere vorliegt, ist die Prognose dagegen verhältnismäßig gut. Die Mortalitätsziffern dieser letzterwähnten Untergruppe sind besser als die des gesamten anurischen Materials (Tab. IV).

Tabelle IV

Gesamtmaterial

Krankenmaterial	Anzahl der Fälle	Mortalität % (Zahl der Fälle)
Anurie bakteriämischen-toxinämischen Ursprungs	87	49,4 (43)
Symmetrische Nierenrindennekrose	8	100,0 (8)
Schockniere	56	32,1 (18)
Akute Aufflackerung einer chronischen Nierenkrankheit	23	74,0 (17)

Aus pathogenetischem Standpunkt kommen bei Nierenrindennekrose glomeruläre Ischämie, Kapillarstase, intravaskuläre Koagulation usw. in Frage.

Unseres Erachtens handelt es sich bei durch bakteriämischen-toxinämischen Schock verursachter Oligo-Anurie um dieselben pathogenetischen Faktoren wie bei der Schockniere, d. h. um die Veränderung des Nierenkreislaufes. BÁLINT [1] wies darauf hin, daß bei Versuchstieren in der postischämischen Phase die Nierendurchblutung verhältnismäßig unverändert ist, die Glomerularfiltration sich dagegen in bedeutendem Maße vermindert. Seiner Ansicht nach ist für die Erscheinung in erster Reihe die Konstriktion des Vas afferens verantwortlich, woraus gefolgert wird, daß im Zustandekommen der Oligo-Anurie der verminderten Filtration eine ausschlaggebende Rolle zukommt.

Wir sind uns darüber im klaren, daß die tierexperimentellen Ergebnisse auf die menschliche Pathologie nur mit dem nötigen Vorbehalt übertragen werden können, es dürfte jedoch angenommen werden, daß sich bei Menschen im Falle von Schock — auch den bakteriämischen-toxinämischen Schock inbegriffen — ähnliche Prozesse abspielen, wie bei den Versuchstieren.

Bei der akuten Aufflackerung chronischer Nierenkrankheiten konnte die Ursache der Anurie histologisch teilweise geklärt werden. Die akute Verkleinerung der bereits vorangehend kleiner gewordenen Glomerulumoberfläche (Glomerulitis) sowie das entzündliche Ödem des Interstitiums tragen zwar in bedeutendem Maße zur Entwicklung der Oligo-Anurie bei, unserer Ansicht nach ist aber auch der erwähnten intrarenalen Kreislaufveränderung — die zur Verminderung bzw. zum vorübergehenden Aufhören der Glomerularfiltration führen kann — eine wesentliche Bedeutung beizumessen. In Anbetracht dessen jedoch, daß in der Frühphase der Oligo-Anurie, bei den sich in schwerem Zustand befindlichen Patienten die Durchführung diesbezüglicher Untersuchungen nicht ratsam ist, kann das Vorhandensein dieser funktionellen Veränderung nicht bewiesen werden.

Die angeführten Angaben sprechen dafür, daß sich auf Wirkung von Bakteriämie-Toxinämie Oligo-Anurie entwickeln kann. Im Hintergrund steht seltener eine symmetrische Nierenrindennekrose, in der Mehrzahl der Fälle kommt jedoch dieser schwere Zustand infolge der akuten Aufflackerung eines mit oder ohne Schock verlaufenden chronischen Nierenprozesses — in erster Reihe chronischer Pyelonephritis — zustande.

Die therapeutischen Richtlinien können in 2 Punkten zusammengefaßt werden: 1. Behebung bzw. Heilung der auslösenden Ursache und 2. Behandlung der Oligo-Anurie an einer Kunstnierenstation, worunter komplexe antiurämische Therapie und nötigenfalls Hämodialyse zu verstehen ist.

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FUNCTIONS OF THE KIDNEY AFTER LIGATION OF THE RENAL ARTERY

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After ligation of the renal artery in dogs, the kidney has been found to recover its functions to a certain extent, provided there is a sufficient collateral blood flow. The formation of an adequate collateral blood supply requires the presence of an intact contralateral kidney within a period of approximately three months. Removal of the intact kidney at the end of this period definitely favours the development of a collateral blood flow providing for the necessary renal blood supply.

The reduced renal blood supply was found to be responsible for the arterial hypertension the persistence of which beyond the critical period of three months has deleterious consequences. The dogs whose right kidney was removed four to six months after ligation of the left renal artery, died without exception.

The observation that after experimental ligation of the renal artery instead of a uniform necrosis macroscopically discernible areas of healthy parenchyma alternating with necrotic or ischaemic patches appear over the whole medulla and cortex, has drawn attention to the importance of the collaterals in renal blood supply (HARTWICH [14], WOLF and HEISEN [22], DAVIS [20]). According to SHEEHAN and DAVIS [20], necrosis resulting from total ischaemization of the kidney is patchy in character. Demarcation of the ischaemic areas from those supplied with blood is demonstrable as early as 4 hrs after the interception of renal blood flow. At 24 hrs the red patches are alive whereas the ischaemic areas have undergone successive infarction. The patchy character of necrosis is generally ascribed to the special blood supply of the kidney, operating outside the main channel of the renal artery, through the collateral system.

The aim of the present study was to investigate the functions of the kidney after ligation of the renal artery. Our earlier finding that the collateral blood supply of the kidney is sufficient for a more or less adequate renal function, has been reported previously (5).

Material and methods

Mongrel dogs of both sexes were used. The intervention was performed under sterile conditions, in hexobarbital anaesthesia. By the midline approach, the left renal artery was ligated 10 to 13 mm away from the hilus and the wound was closed. One group (a) of 7 dogs was intended for later acute experiments, in another group (b) of 19 dogs the right kidney was

removed 42 to 186 days after ligation of the left renal artery. The survivors were used for acute experiments. 9 dogs of this group (c) were kept in metabolic cages and urine output, the concentrations of creatinine, carbamide, sodium, and osmolarity of urine and blood serum were estimated daily, and the nonprotein nitrogen value, every other day.

The acute experiments were performed under chloralose anaesthesia. The left kidney with the ligated renal artery was exposed from a midline incision, the renal vein was carefully prepared and connected with the left jugular vein by means of a plastic tube [4]. By the T-branch of the tube the blood flowing from the vein was measured (direct RBF). Urine was collected by an ureter catheter. The necessary concentrations of exogenous creatinine, inulin and PAH were ensured by the usual infusion technique. Glomerular filtration rate was computed from RPF calculated by means of the haematocrit and the E_{in} (GFR = RPF $\times$ E_{in} or E_{cr}). Oxygen concentration in the blood of the renal vein and artery was measured by an Atlas oxymeter, the osmotic concentration of the urine by an osmometer, the Na-concentration by flame photometry. The normal values having been recorded, Tm_{PAH} was determined at raised plasma PAH concentration. For details, see [6, 7, 8].

The acute experiment was completed by ligating the aorta 2 cm above and 5 cm below the origin of the renal artery and by injecting india ink, in one case polyvinylpyrrolidone [18] into the latter vessel. The kidney was then carefully isolated and subjected to morphologic and histologic study.

Results

a) Acute experiment in 7 dogs on the 7th, 13th, 20th, 50th, and 106th day, respectively after total ligation of the left renal artery, the right kidney being left intact. The results are seen in Table I. Left RBF with the renal artery

Table I

Data for left kidney, 7 to 106 days after arterial ligation, with functioning right kidney

No.	Day after ligation	Renal weight g		Blood pressure mm Hg	RBF	GFR	V/min	E_{cr}	E_{in}	E_{PAH}	AV_{O_2}	Q_{O_2}
		left	right		ml/min/100 g							
77/64	7	30.3	37.5	140	48	3	0	0.00	0.07	0.10	3.4	1.63
78/64	7	29.2	39.1	130	80	2	0	0.00	0.04	0.00	1.5	1.20
85/64	13	18.4	22.8	120	104	4	0	0.06	0.07	0.09	0.9	0.94
88/64	13	28.8	30.5	115	100	0	0	0.01	0.00	0.04	0.8	0.80
91/64	20	25.4	29.5	115	85	0	0.07	0.02	0.00	0.04	0.3	0.25
15/65	50	18.2	31.7	140	37	0	0.03	0.04	0.02	0.00	1.2	0.44
37/65	106	13.5	57.2	130	34	0	0.07	0.03	0.03	0.12	1.2	0.41

ligated (pro 100 g kidney weight) was between 1/10 and 1/4 of the normal RBF. Filtration, excretion, and urine output were minimal. The kidneys showed atrophy. Though the india ink did not fail to reveal the presence of collaterals and of a patent communication prevalently in the subcortical areas (glomerules filled with india ink), there was parenchymal degeneration with extensive destruction of nephrons which showed no sign of any function (Fig. 1).

b) Right nephrectomy 42 to 186 days after ligation of the left renal artery in 19 dogs. Table II presents the intervals in days between the two interventions and the fate of these animals left with one kidney whose artery

had been ligated. When the interval between the two interventions was shorter than 80 days or longer than 100 days, after removal of their right kidney the animals died with uraemia in 6 to 11 days (one animal in 30 days). The six

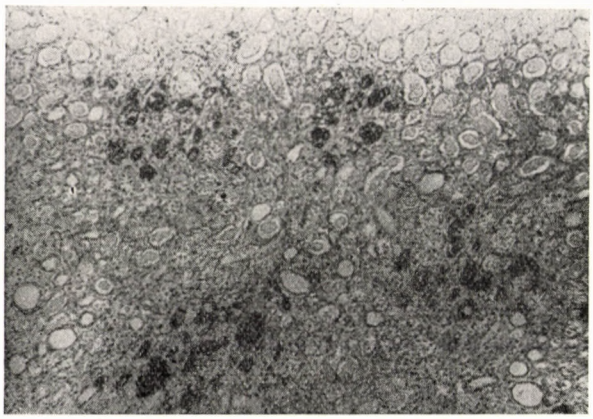

Fig. 1. Atrophy of left kidney 50 days after ligation of the renal artery; intact right kidney

Table II

Survival and mortality after ligation of left renal artery and removal of right kidney

No.	Interval in days between the two interventions	Outcome
33/64	42	death on 6th day
34/64	49	death on 8th day
35/64	71	death on 8th day
116/65	81	survival
64/65	87	survival
35/65	93	survival
47/65	93	survival
121/66*	99	survival
36/65	99	survival
43/65	101	death on 11th day
56/65	102	death on 7th day
53/65*	111	death on 8th day
127/66*	117	death on 8th day
30/65*	146	death on 6th day
39/65*	153	death on 9th day
35/65*	170	death on 9th day
75/65*	179	death on 29th day
63/65*	183	death on 7th day
32/65*	186	death on 9th day

animals which had been subjected to right nephrectomy 80 to 100 days after ligation of the left renal artery, survived without exception. In 4 of these, nephrectomy was followed by azotaemia in the range of 100 mg per 100 ml in 2 animals, declining to normal values in 2, whereas in the remaining 2 dogs NPN was normal throughout (Fig. 2).

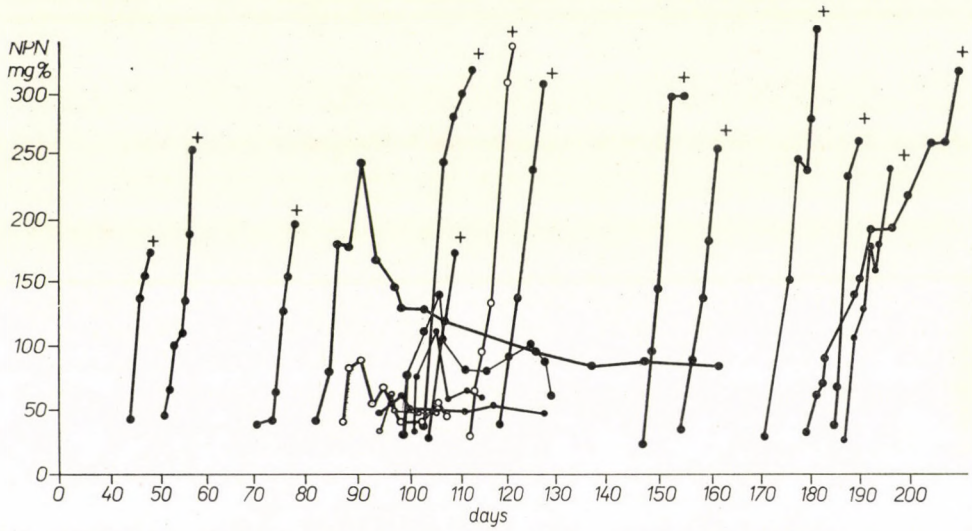

Fig. 2. NPN in the first days after ligation of the left renal artery and rigth nephrectomy Abscissa: interval between the two interventions. Ordinate: NPN, mg per 100 ml

Those animals were considered survivors which were alive with normal NPN-values on the 14th day or with elevated NPN-values on the 30th to 80th day after nephrectomy. These animals were subjected to an acute experiment between the 14th and 80th day (Table III). The five successful experiments resulted in the following findings. Blood pressure was elevated, RBF was $^1/_2$—$^1/_3$ of the normal; E_{creat}, E_{in} and E_{PAH} were fairly preserved in contrast to a reduced PAH-secretion (Tm approximately 5); diuresis was normal whereas osmolarity of the urine was low. The values for the arterio-venous O_2 difference have been corrected according to DOLE et al. [11]. Renal O_2-uptake (Q_{O_2}) was in the normal range [7].

Recording of blood pressure over the whole acute experiment was begun preoperatively. It can be seen from columns 2 and 3 of Table III that the values of 185 to 210 mm Hg found before preparation had fallen by the time of the acute experiment to between 140 and 160 mm Hg on account of the isolation of the renal vein or, more precisely, in consequence of the haemorrhages involved by its isolation. But even these values exceeded the normal range.

At autopsy, the kidney whose artery had been ligated was literally fused with its environment; it displayed an abundant vascular network with dilated

Table III

Renal function in the survivors

	116/65	64/65	35/65	47/65	121/66	36/65
Blood pressure						
before intervention	210	185	190	205	210	200
after intervention	160	165	150	145	140	—
RBF ml/min	217	273	324	266	410	
Ht	20	37	38	37	27	
RPF ml/min	174	172	201	167	298	
GFR ml/min	24	21	36	30	48	
E_{creat}	0.10	0.12	0.08	0.16	0.16	
E_{in}	0.14	—	0.12	0.18	—	
E_{PAH}	0.30*	0.59	0.56	0.66	0.44**	
V/min/ml	0.86	0.50	0.48	2.71	1.40	
Tm_{PAH}	—	4.8	4.9	5.0	4.9	
Hb g per 100 ml	7.9	12.6	14.0	14.0	9.3	
AVD_{O_2} vol%	1.7	1.6	1.5	1.7	1.4	
Q_{O_2} ml	3.70	4.37	4.85	4.53	4.16	
$U_{Na} \cdot V$	26	66	30	510	220	
U/P_{osm}	1.3	2.9	1.0	1.8	1.8	
NPN mg per 100 ml	104	42	40	36	62	70
Renal weight, g	19.4	45.8	60.2	35.0	16.2	21.4

acute experiment failed

* $P_{a\text{-}PAH}$ over 5 mg per 100 ml ** $P_{a\text{-}PAH}$ over 7 mg per 100 ml

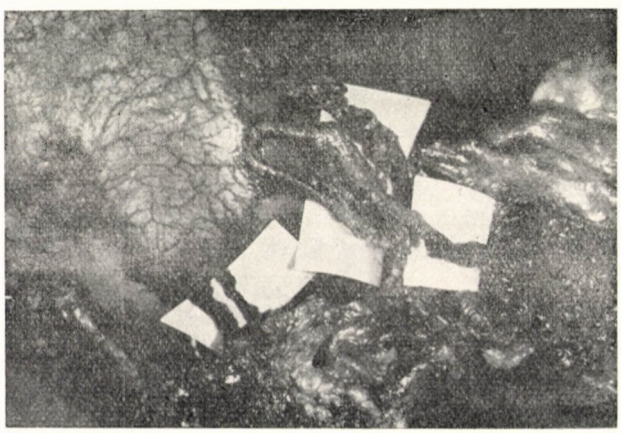

Fig. 3. Left kidney after ligation of its artery. Collaterals originating from the mesenterial system (in situ, in vivo; animal No. 64/65)

Fig. 4. Collaterals and vascular network of the ligated left kidney after intraarterial PVP injection (animal No. 116/65)

Fig. 5. Patchy necrosis after ligation of renal artery (animal No. 47/65)

capsular vessels. The ureter was thickened. The kidneys weighed less than normal but for one case where they weighed more. The collaterals seen in Fig. 3 originated from the mesenterial system (No. 64/65). Fig. 4 shows the collaterals and the vascular network of animal No. 116/65 after injection of polyvinyl pyrrolidone. The necrotic patches alternating with intact areas in animal No. 47/65 are seen in Fig. 5.

c) 9 animals (marked* in Table II) were kept after right nephrectomy in metabolic cages. One of these animals (No. 121) survived, one (No. 29) died with uraemia on the 29th day, the other 7 between the 6th and 11th days.

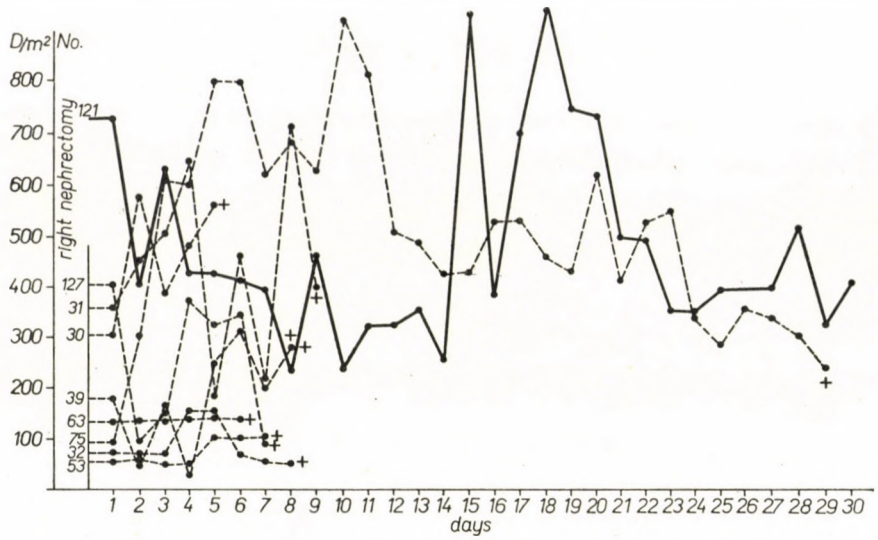

Fig. 6. Daily diuresis per sq. m body-surface in one group of animals with ligated left kidney
Abscissa: number of days after right nephrectomy

According to Fig. 6, in four animals (Nos 127, 30, 31, 75) which had died, urinary output was similar to that of the survivors during the same period whereas the remaining four (Nos 53, 32, 63, 39) were definitely oliguric. In all of the animals which had died, creatinine and carbamide excretion was low and NPN rose rapidly (Fig. 2). The osmotic quotient of blood and plasma was around 1, in other words, the kidney was hypo- or asthenuric. Comparative data recorded over 28 days in one of the survivors and in one of the lethal cases, are presented in Fig. 7.

In the dogs which had died of renal failure, the kidneys with ligated artery showed gross atrophy and weighed between 8 and 16 g. They were fused with the adjacent tissues. Their abundant collateral network was of a cobweb-like delicacy. Apart from slight quantitative differences, the histologic pattern was uniform, its main features being cortical atrophy and thinning in contrast to fairly preserved subcortical areas with numerous normal, often gigantic juxtamedullar glomerules arranged in typical strings or groups, and to intact medullary areas (Fig. 8).

Discussion

The collateral system supplying the kidney in human beings, dogs, and rabbits [1, 9, 10, 16] has the following constituents.

a) Direct lumbar branches of the aorta running behind the kidney and entering this organ in the hilum, parallel with the renal artery; *b)* vessels which accompany the ureter and supply the connective and fat tissues of the

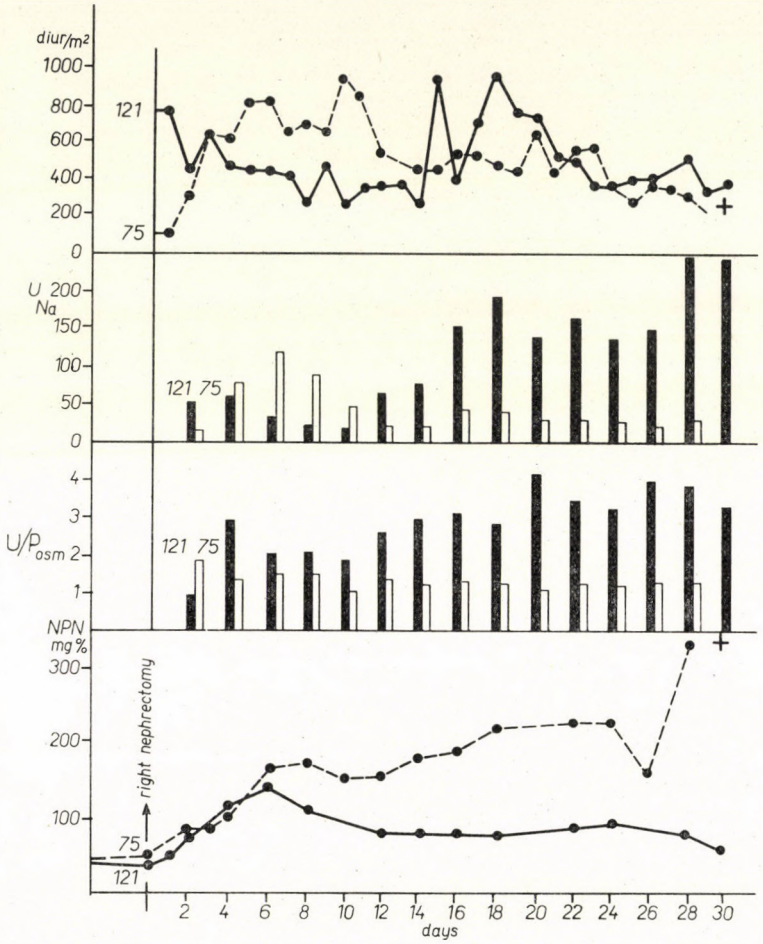

Fig. 7. Correlation of data of survivors with those of the lethal cases

renal pelvis as well as the perihilar region of the cortex. These vessels communicate with the arcuate artery and supply large areas of the renal parenchyma; *c)* an abundant capsular network originating from the spermatic, suprarenal, phrenic (arcus exorenalis), superior, and inferior mesenteric arteries. The different distribution of the areas left intact after ligation of the renal artery is due to individual variations in these collaterals [20]. The investigations of WOLF and HEISEN [22] into the blood supply of these areas have shown that one week after ischaemization the blood leaves the renal vein at a rate of 10 to 20 drops per minute which corresponds to 20 per cent of the normal flow. On the evidence of other studies the blood leaving the kidney on the fifth day after interception of its blood supply corresponds to 1/10 to 1/4 of that of the other side and urinary excretion ceases at the affected side [23]. According to

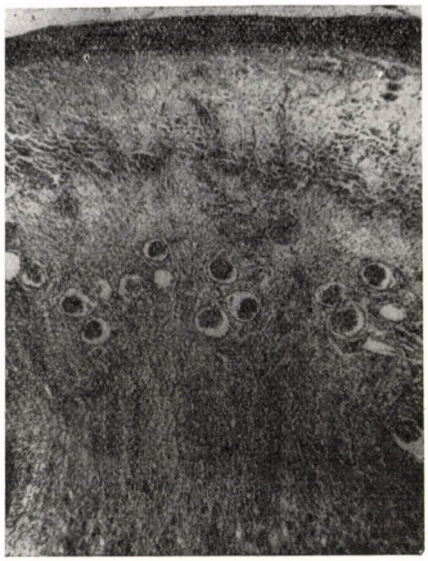

Fig. 8. Cortical atrophy with hypertrophic juxtamedullar glomerules of animal No. 30/65, died 179 days after ligation of the left renal artery and 9 days after right nephrectomy

SHEEHAN and DAVIS [20] one third to one half of the renal parenchyma may be kept alive by the collateral blood supply.

Results obtained in our group *a)* showed that, after ligation of its renal artery, the left kidney receives a minimal blood supply between the 7th and 106th day after ligation, and the organ undergoes gradual atrophy and shows no sign of any function during the entire period.

If right nephrectomy was performed 80 to 100 days after ligation of the left renal artery, a collateral system providing for approximately two thirds of the normal renal functions had time to develop and all of the six animals survived [5, 12]. Resumption of renal function reflecting an adequate collateral blood supply was demonstrable in six cases after ligation of the renal artery. These results seem to suggest that development of an adequate collateral blood supply requires a certain period of time, since no animal survived if the interval between the ligation of the left renal artery and the right nephrectomy had been less than 80 days.

If an 80-day-interval allows sufficient time for the collaterals to develop to the extent of providing for the functions of a kidney whose blood supply through the renal artery has been cut off, why then do the animals succumb to nephrectomy of the contralateral side if the interval separating it from the ligation of the left renal artery is as long as 101 to 186 days? The facts revealed by the investigations of GOLDBLATT et al. [13] into the mechanism of induced hypertension permit to assume that the reduced blood flow associated with the ligation of the renal artery elicits an increased production of renin accounting

for the maintenance of hypertension. In 5 acute experiments performed in this series, blood pressure ranged between 180 and 210 mm Hg between the 14th and 80th days after removal of the right kidney. In the light of the GOLDBLATT-mechanism the fact that the collaterals are capable of supplying the kidney after ligation of the renal artery appears to be fully consistent with the eventual production of hypertension.

On the evidence of PICKERING's rabbit experiments [19], the consequences of hypertension are related to its duration. If it has persisted longer than 50 days, it is beyond the stage when contralateral nephrectomy is of any use, as according to PICKERING by this time its maintenance has been taken over by extrarenal mechanisms. HUBER [15] has shown that in the rat hypertension takes 65 days to become irreversible.

In conformity with data in the literature, the present results clearly incriminate the reduced renal blood supply for the maintenance of arterial hypertension. The critical period beyond which arterial hypertension is bound to affect renal function may be estimated at three months. If the hypertension persists for four to six months — as it supposedly did in our cases with fatal outcome — then it is well possible that it accentuates the tendency to constriction of renal vessels, particularly in a previously damaged kidney.

In this manner the animals which had been nephrectomized 101 to 186 days after ligation, must have reached a stage of arterial hypertension where despite an adequate collateral blood supply, arteriolar damage and cortical atrophy have so far advanced that contralateral nephrectomy must have led to fatal renal failure. We are thus inclined to ascribe the deaths in this group to arterial hypertension and to its extrarenal consequences.

Further investigations into the nature of the hypertensive condition induced by the ligation of the renal artery are in course.

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THE MECHANISM OF ELECTROCARDIOGRAPHIC CHANGES IN THYROTOXICOSIS

By

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An analysis of the ECG changes in thyrotoxicosis and the relationship between the ECG and some clinical and haemodynamic factors has been performed. The results indicate that in addition to thyroid overactivity other factors also contribute to the ECG changes in patients with thyrotoxicosis.

The influence of thyroid overactivity on the electrocardiogram has often been studied [7, 8, 10, 15, 19]. The results, however, are controversial. One of the most discussed questions is whether the cause of the ECG changes in thyrotoxicosis is solely the increased thyroid activity or whether some other factors also have a part in these changes [1, 8, 19].

The purpose of the present paper was to investigate the relationship between the ECG and some clinical and haemodynamic factors. Therefore, the influence on the ECG of the patients' age, duration of the illness, body mass, blood pressure and the sympathetic system has been studied in patient with thyrotoxicosis.

Material and methods

We have investigated 36 patients with thyrotoxicosis, diagnosed on the basis of the clinical picture and laboratory findings, including ^{131}I uptake. Four ECGs were recorded in every case. The first curve was taken in the supine position, the second one in the upright position, during the first minute. The next two curves, in supine and upright position, were recorded 90 minutes after adrenergic blockade. Sympathetic activity was inhibited with the beta-adrenergic blocking drugs pronethalol [3] and propranolol* [2] in doses of 300—400 mg and 40 mg, respectively, administered orally.

In every patient, the following ECG parameters were analysed: heart rate, PQ interval, the amplitude of P_2, T_2 R_{V5} and the sum of $S_{V1} + R_{V5}$. The data were correlated with the patient's age, duration of the illness, ponderal index (body mass) [13] and blood pressure. Statistical significance was computed with Student's T test.

Results

In Tables I and II and Figure 1 the clinical data and the influence of postural changes before and after adrenergic blockade are presented. From Fig. 1 and Table I it is evident that the adrenergic blockade in the supine posi-

* Kindly supplied as Alderlin and Inderal by Imperial Chemical Industries, Ltd.

tion decreased the heart rate from 108/min. to 91/min. ($p < 0.01$), and that the orthostatic increase in heart rate before the application of beta-adrenergic blocking drugs was 12/min. and after the inhibition of the sympathetic system, 9/min. The increase in heart rate in the upright position before and after adrenergic blockade was significant statistically ($p < 0.01$). Figure 1 indicates

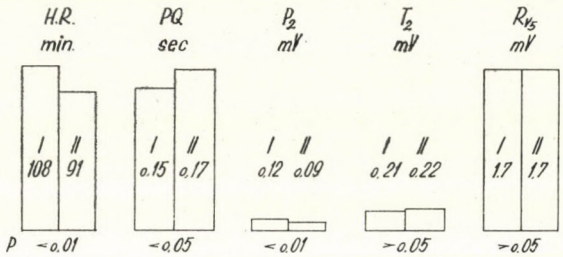

Fig. 1. Effect of adrenergic blockade in the ECG. I. Before adrenergic blockade; II. After adrenergic blockade

Table I

Clinical data

	Mean	Ranges	
Age	47.5	23.0	69.0
Ponderal index	12.8	11.0	13.8
*Duration of illness	38.4	2.0	140.0
Systolic blood pressure	152.0	120.0	210.0
Diastolic blood pressure	88.0	70.0	130.0
Mean blood pressure	109.0	90.0	156.6

* Expressed in months

that adrenergic blockade did not completely abolish the tachycardia in patients with thyrotoxicosis. At the same time, a significant correlation was found between resting heart rate and the effect of adrenergic blockade ($r = 0.50$, $p < 0.01$), which means that the most pronounced effect of adrenergic blockade occurred in patients in whom the heart rate was the highest.

The PQ interval in the supine position was 0.155 sec. and adrenergic blockade prolonged it to 0.17 sec. ($p < 0.05$). In the upright position before sympathetic inhibition there was a shortening of the PQ interval (minus 0.015 sec.) and after adrenergic blockade a shortening amounting to 0.02 sec. Both these changes were significant statistically ($p < 0.01$).

There are many factors which influence the PQ interval. One of these is the heart rate. Its increase leads to a shortening of the PQ interval. In Fig. 2

Table II

Influence of postural changes on the electrocardiogram before and after adrenergic blockade

		Before adrenergic blockade		After adrenergic blockade	
		Supine pos.	Upright pos.	Supine pos.	Upright pos.
Heart rate	M±				
	SEM	108±2.50	120±2.66	91±1.98	100±1.91
	P	<0.01		<0.01	
PQ interval	M±				
	SEM	0.155±0.0046	0.140±0.0039	0.170±0.0041	0.150±0.0039
	P	<0.01		<0.01	
Height of P_2	M±				
mV	SEM	0.12±0.0081	0.16±0.0123	0.09±0.0065	0.12±0.0097
	P	<0.05		<0.05	
Height of T_2	M±				
mV	SEM	0.21±0.0160	0.13±0.0160	0.22±0.015	0.18±0.0160
	P	<0.01		>0.05	
Height of RV_2	M±				
mV	SEM	1.70±0.0790	1.50±0.0930	1.70±0.067	1.50±0.0820
		>0.05		>0.05	

is presented the relationship between heart rate and PQ interval before and after adrenergic blockade in the supine position. Fig. 2 indicates that in 11 patients before and in 7 patients after the adrenergic blockade there was a prolongation of the PQ interval. Otherwise, we found no statistically significant correlation between heart rate and PQ interval neither in the supine nor in the upright position. The results confirm the assumption that in some patients with thyrotoxicosis there is a relative prolongation of the PQ interval.

According to several papers [14, 15, 18, 19] there is an increase in the amplitude of $P_{2,3}$ in patients with thyrotoxicosis. In our group the mean value for P_2 was 0.12 mV. Adrenergic blockade decreased it significantly to 0.09 mV ($p < 0.01$). During the postural changes before and after adrenergic blockade there was a statistically significant increase in the voltage of P_2 ($p < 0.05$). The relationship between sympathetic activity and the amplitude of P_2 is also evident from the significant correlation between the decrease in amplitude of P_2 after sympathetic inhibition and the resting amplitude of the P_2. The most pronounced effect of adrenergic blockade was seen in patients in whom the voltage of P_2 was the highest ($r = 0.65$, $p < 0.01$). The correlation between heart rate and the amplitude of P_2 was not significant ($r = 0.29$, $p > 0.05$).

Thyroid overactivity is known to influence the T wave [7, 15, 18, 19]. In our patients the mean amplitude of T_2 was 0.21 mV. Adrenergic blockade had no effect on the voltage of T_2. During orthostasis there was a significant decrease of the amplitude of T_2 ($p < 0.01$) and adrenergic blockade prevented any significant flattening of the T_2 in the upright position. Other factors may

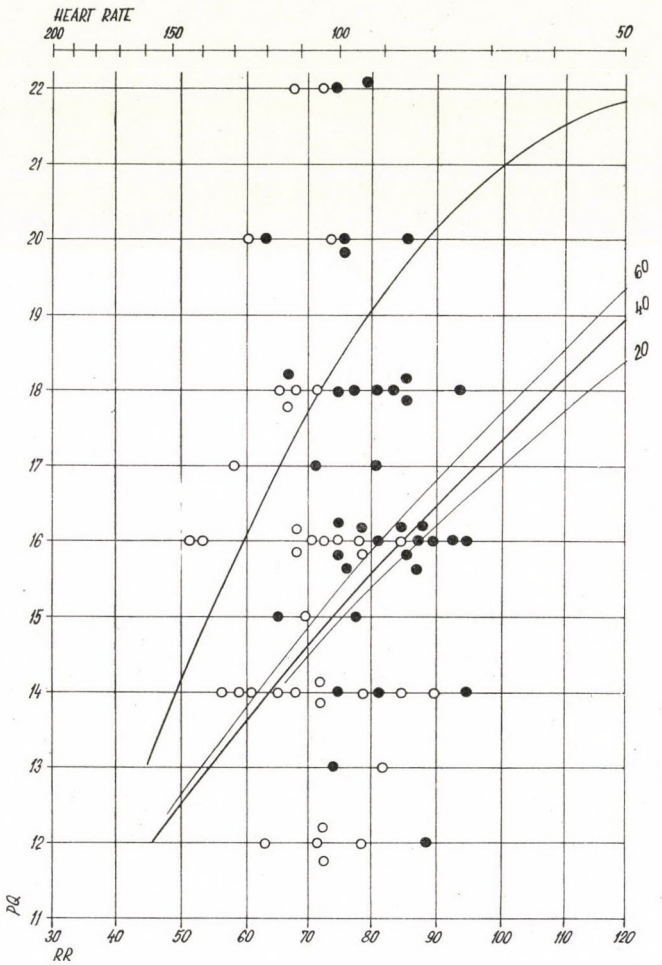

Fig. 2. ○ Before adrenergic blockade • After adrenergic blockade

also cause T wave changes in patients with thyrotoxicosis. In our group the correlation between age and the amplitude of T_2 was only significant statistically. With age, the T wave became smaller ($r = -0.33$, $p < 0.05$).

The voltage of the R_{V5} wave is increased in some patients with thyrotoxicosis [15, 18]. Adrenergic blockade and postural changes had no effect on

the amplitude of R_{V5}. On the other hand, there was a significant correlation between the amplitude of R_{V5} and the systolic blood pressure. On increasing blood pressure there was an increase in the amplitude of R_{V5} ($r = 0.36$, $p < 0.05$). Similarly, there was a significant correlation between the sum of $S_{V1} + R_{V5}$ and systolic blood pressure ($r = 0.33$, $p < 0.05$). The relationship between the ponderal index and the R_{V5} wave was not significant statistically ($r = 0.02$, $p < 0.05$).

Discussion

Tachycardia is one of the constant symptoms of thyrotoxicosis. The acceleration of the heart rate may have two causes. The first one is the thyroid overactivity and the second the increased activity of the sympathetic system. The participation of this second factor is not generally accepted [4, 5, 9, 11, 17, 20]. From our results it may be concluded that the sympathetic system has a part in thyrotoxic tachycardia and that the sympathetic acceleration of the heart rate varies from patient to patient.

Thyroid overactivity increases the amplitude of the P_2 wave [14, 15, 18, 19]. This is due either to the increased sympathetic activity or to the tachycardia itself. In our patients there was no significant correlation between heart rate and the amplitude of P_2. On the other hand, there was a significant decrease in the amplitude of P_2 after sympathetic blockade and a significant correlation between the resting amplitude of P_2 and the decreasing effect of the adrenergic blockade. These observations support the assumption that the cause of the increase in the amplitude of P_2 rests with the sympathetic system. The mechanism by which these changes are brought about, remains unknown.

The influence of thyrotoxicosis on the T wave has often been studied in recent years [7, 15, 18, 19]. We have shown [16] that in young thyrotoxic patients the T wave is often increased. From the present results it may be concluded that with increasing age a flattening of the T_2 wave occurs. It is not known whether this was due to the age itself or rather to ischaemic heart disease often encountered in older patients.

In some patients with thyrotoxicosis there is an increase in the voltage of the R_{V5} wave [15, 18]. This may be caused either by a hypertrophy of the left ventricle or by the thinness of the thoracic wall. The non-significant correlation between ponderal index and the amplitude of R_{V5}, and the significant relationship between the amplitude of R_{V5} and systolic blood pressure support the assumption that the increased amplitude of R_{V5} may be caused by the hypertrophy of the left ventricle.

As to the practical importance of the results, the question arises whether or not, beta-adrenergic blocking drugs are indicated in thyrotoxicosis. Our results support the opinion of those who recommend a simultaneous adminis-

tration of antithyroid drugs and beta adrenergic blocking drugs [4, 9, 17]. We believe that, particularly at the beginning of treatment, when antithyroid therapy is not yet effective, beta adrenergic blockade should be applied. According to our preliminary results there is yet another indication for beta-adrenergic blocking drugs. We have namely achieved some improvement with propranolol in patients, who received triiodothyronine and thyroidine either as a treatment of hypothyroidism or of goitre.

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STARLING'S LAW OF OEDEMA PRODUCTION

ITS MATHEMATICAL ANALYSIS FROM HAEMO-LYMPHODYNAMIC ASPECTS

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STARLING's law assigns the same oedematogenic effect to the increase in capillary pressure and to the reduction in colloid osmotic pressure.

Taking into account the lymphodynamic aspects of oedema production, it is claimed that in systemic venous hypertension the increase in capillary pressure represents an oedematogenic potency twice as great as the reduction in colloid osmotic pressure.

STARLING's law in its classical form as well as with its modification by the present authors is definable in equations.

On the basis of the pertaining data of KORÁNYI and FARKAS, a new mathematical correlation has been found to substantiate the claim that in systemic phlebohypertension the oedematogenic potency of capillary pressure is about twice that of colloid osmotic pressure.

STARLING's classical law concerning the equilibrium between capillary pressure and colloid osmotic pressure of plasma proteins may be defined by the equation

$$K_s - K_i = P_c - P_i \tag{1}$$

where

K_s represents the colloid osmotic pressure of blood serum;

K_i, the colloid osmotic pressure of interstitial fluid;

P_c, the capillary pressure;

P_i, the interstitial (tissue) pressure.

In a state of equilibrium satisfying this equation, about 20 litres of fluid are filtered in 24 hrs through the arterial end of the capillaries into the interstitium in a healthy adult. 16 to 18 litres of this are reabsorbed through the venous end of the capillaries and 2 to 4 litres are returned into the circulation as lymph.

The following equation formulated by LANDIS and PAPPENHEIMER (1963) expresses the same relationships as equation (1) except for the introduction of a filtration coefficient

$$FM = k(P_c - K_s - P_i + K_i) \tag{2}$$

where

FM represents the filtration fluid flow, and

k represents the coefficient.

The present study deals with the oedematogenic effect of the changes in P_c and K_s.

Whether analyzing phlebohypertonic oedema due to an elevation of P_c, or hypooncotic oedema due to a reduction of K_s, both will presumably influence K_i and P_i to exactly the same extent in the acute experiment. Therefore, equation (1) may be simplified as follows:

$$K_s = P_c \tag{3}$$

expressing the fact confirmed by measurements that, in every animal species hitherto studied, capillary pressure and colloid osmotic pressure of plasma proteins are practically equal under normal conditions.

It follows from equation (3) that

$$a_k + a = a_p + a \tag{4}$$

$$a_k + 2a = a_p + 2a \tag{5}$$

$$a_k + na = a_p + na \tag{6}$$

where

a_k and a_p represent the arbitrary values of K_s and P_c.

In other words, elevation of K_s and of P_c to the same extent leaves the equilibrium of fluid passage unaffected and leads to no fluid retention.

It follows from equation (3) that phlebohypertonic oedema of increasing severity is produced if

$$K'_s < P_c + a < P_c + 2a < \ldots < P_c + (n-1)\,a < P_c + na \tag{7}$$

It also follows from equation (3) that hypooncotic oedema of increasing severity is produced if

$$P'_c > K'_s - a > K_s - 2a > \ldots > K'_s - (n-1)\,a > K'_s - na \tag{8}$$

K'_s and P'_c representing the arbitrary basic values of K_s or P_c.

The foregoing theoretical considerations implicate that oedema is of precisely the same severity whether resulting from shifts in fluid balance fitting equation (7) or (8).

The condition in which a given elevation of venous pressure is associated with precisely the same reduction in colloid osmotic pressure, obviously doubles the severity of oedema:

$$K' - na < K_s - (n-1)\,a < \ldots < K'_s - 2a < K'_s - a < P'_c + a < P'_c + \\ + 2a < \ldots < P'_c + (n-1)\,a < P'_c + na \tag{9}$$

(see Fig. 1).

These considerations based on STARLING's law leave the fact out of account that in hypooncotic states the "safety valve" function of the lymphatic bed asserts itself unhindered and resists fluid retention until the extreme point is reached where excessive filtration has led to an overproduction of intersti-

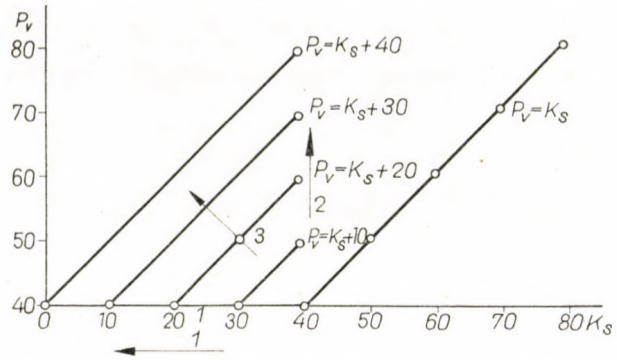

Fig. 1. Right side: Equation (3). Left side: Equations (7) and (8). P_c (capillary pressure) has been replaced by P_v (venous pressure) this being more convenient to measure. P_v and K_s are assumed to be 40 cm H_2O, a to be 10 cm H_2O. The arrows indicate the direction of the shift from oedema-free to oedematous conditions

tial fluid which surpasses the transporting capacity of the lymphatics. A local increase in capillary pressure involves the same conditions. In contrast, in systemic phlebohypertension, the very same increase in venous pressure which has overthrown STARLING's equilibrium in the capillary system, prevents the drainage of lymph into the venous system (2, 3, 4, 7). In opposition to the general view, we must therefore regard an increased K_s in the presence of systemic phlebohypertension as an oedematogenic force twice as great as a reduced K_s. Accordingly, an equilibrium in systemic phlebohypertension must satisfy the following equation,

$$a_{P_1} + a = a_{K_1} + 2a \tag{10}$$

When representing these correlations graphically, a_P' and a_K' are assumed to be 40 and a to be 10 cm H_2O, then:

$$P_1 = 40 + a \tag{11}$$

$$K_1 = 40 + 2a \tag{12}$$

It follows that

$$a = P_1 - 40$$

and

$$a = \frac{K_1 - 40}{2}$$

Therefore

$$P_1 - 40 = \frac{K_1 - 40}{2}$$

and

$$P_1 = \frac{K_1 - 40}{2} + 40$$

i.e.

$$P_1 = \frac{K_1}{2} + 40 - \frac{40}{2}$$

or

$$P_1 = 0.5\ K_1 + 20 \tag{13}$$

Equations (10) and (13) are represented in Fig. 2.

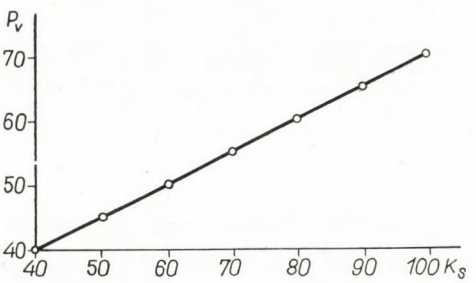

Fig. 2. Representation of equation (10) a'_p and a'_k being 40 cm H_2O, a 10 cm H_2O

In order to decide whether the equation corresponding to STARLING's law

$$K_s = P_c \tag{3}$$

or that based on our hypothesis

$$P_c = 0.5\ K_s + 20$$

is valid in generalized phlebohypertension in humans, we have made use of the material of FARKAS (1928) and KORÁNYI (1930). These authors measured the colloid osmotic pressure of plasma proteins and the venous pressures in 110 oedematous and non-oedematous subjects, the former group comprising unselected cases of cardial, renal, and cachectic oedema.* Table I gives a summary of the data obtained; mean values and scatter have been computed by us.

* Colloid osmotic pressure was computed from the serum albumin and globulin values. Venous pressure was measured instead of capillary pressure. Both procedures are still acceptable.

Table I

Mean values and scatter in the 110 cases studied by FARKAS [1] *and* KORÁNYI [4]

Degree of oedema	K_s		P_v		$\Delta\ (=K_s-P_v)$		Number of patients
	Mean	Scatter	Mean	Scatter	Mean	Scatter	
Oedema absent (Ø)	31.0	4.3	6.6	2.8	24.4	4.8	67
Oedema, slight (1)	22.3	6.6	7.2	4.4	15.1	3.6	12
Oedema, moderate (2)	23.9	5.7	8.8	6.6	15.1	4.9	11
Oedema, severe (3)	23.2	7.6	12.5	7.3	10.7	5.4	13
Oedema, massive (4)	27.8	6.0	21.0	7.4	6.8	4.6	7

KORÁNYI represented the data collected by FARKAS geometrically (Fig. 3) and arrived at a defi ition of the correlations between oedema formation, venous pressure and coll id osmotic pressure by two limiting straight lines. By this, he furnished the first exact geometric demonstration of the statement

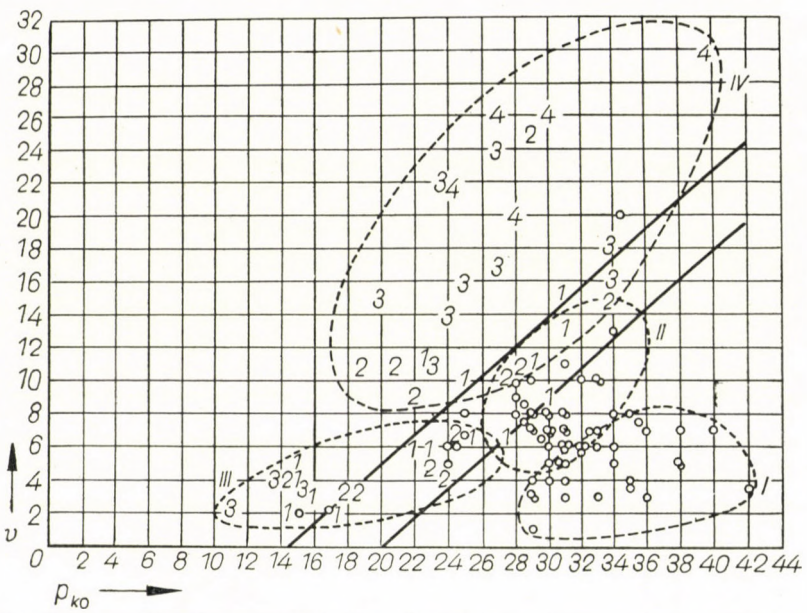

Fig. 3. The FARKAS—KORÁNYI graph in its original form, P_{ko} = colloid osmotic pressure v = venous pressure ([6] and [7])

derived from STARLING's law that the values for venous and the colloid osmotic pressures are interchangeable.

On checking the calculations for the two straight lines we have found that they require a minor correction, i.e.

$$P_v = 0.89\ K_S - 12.9 \qquad (14)$$

and

$$P_v = 0.89\ K_s - 17.8 \tag{15}$$

where

P_v represents the venous pressure.

These two lines may be substituted by an intermediate critical line, its equation being

$$P_v = 0.89\ K_s - 15.3 \tag{16}$$

Going further, we derived from each aggregation of points which KORÁNYI himself had enclosed by limiting lines (1930) (Fig. 3) a straight line defined as follows.

a) Its angle of inclination must be such that its maximal length fall between the perpendiculars drawn from the extreme points of the aggregations.

b) The difference between the mean distances from the points situated at the upper and lower sides should not exceed 0.2 units, i.e.

$$\frac{\sum_{1}^{nf} \Delta V_n(\text{upper})}{n_f} - \frac{\sum_{1}^{n'} \Delta V_n(\text{lower})}{n_a} < 0.2$$

(the line must lie in the middle of the aggregate of points).

Condition *(a)* defines the gradient of the line and condition *(b)* the section it cuts off from coordinate y (Fig. 4).

The equations of the lines thus constructed are,

in the absence of oedema:	$V_0 = 0.34\ P_{ko_0} - 3$	(17)
in minor oedema:	$V_1 = 0.72\ P_{kc_1} - 8.9$	(18)
in moderate oedema:	$V_2 = 0.96\ P_{ko_2} - 12.2$	(19)
in massive and excessive oedema:	$V_{3-4} = 1.01\ P_{ko_{3-4}} - 9.3$	(20)

In the absence of oedema the gradient is 0.34 and increases successively to 1.01. When leaving factor *(b)* out of account, this gives the numerical value of the increase in venous pressure in relation to the colloid osmotic pressure of the plasma.

The gradient of the line satisfying equation (17) which applies to the cases without fluid retention, nearly corresponds to that of equation

$$P_c = 0.5\ K_s + 20 \tag{13}$$

derived by us on the basis of theoretical considerations. This substantiates our claim that venous, i.e. capillary pressure in systemic phlebohypertension is an oedematogenic factor twice as potent as colloid osmotic pressure. Factor *b* figuring in the equations of KORÁNYI and in our equations do not reflect the

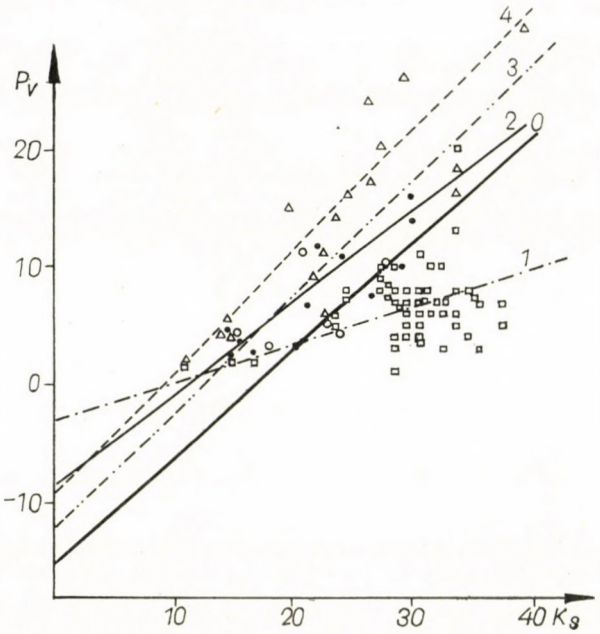

Fig. 4. Representation of the equations derived from the cases of FARKAS and KORÁNYI (equations (17) to (20)

relationships between the capillary and the colloid osmotic pressures. In fact, what this factor expresses in the KORÁNYI—FARKAS equations is an excess of capillary pressure over venous pressure, its value being dependent on the technique employed, but its variations shift the line merely upward or downward without affecting the gradient.

The decisive role of the gradient of the curve, in other words, the twofold oedematogenic effect of venous pressure in systemic phlebohypertension is further supported by the following relationships.

When dividing successively the gradients of the lines corresponding to equations (17), (18), (19), and (20) by the gradient of the line suiting equation (17) it becomes clear that with the increase of oedema the oedematogenic effect of venous pressure gradually increases. From the graph representing the relationships between the degree of oedema and the quotients (Fig. 5) it clearly emerges that in the patients studied by KORÁNYI and FARKAS the oedematogenic effect of venous pressure as compared with that of colloid osmotic pres-

sure, asserts itself more and more as the oedema grows in severity until it has reached a maximum which limits its further increase. This is what happens in excessive oedema.

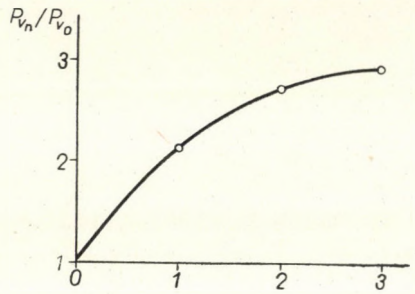

Fig. 5. Correlations between the gradients and the degree of oedema. Abscissa: 0 = oedema absent, 1 = oedema, slight 2 = oedema, moderate, 3 = oedema, severe and massive

In the cases studied by KORÁNYI and FARKAS, the value

$$\Delta = P_c - K_s$$

showed a decrease from the normal mean of 24.4 to 15.1, and K_s an increase from 22.3 to 23.9, in the cases of 1st and 2nd degree of severity alike. If colloid osmotic pressure alone is to determine a slight fluid retention in an originally

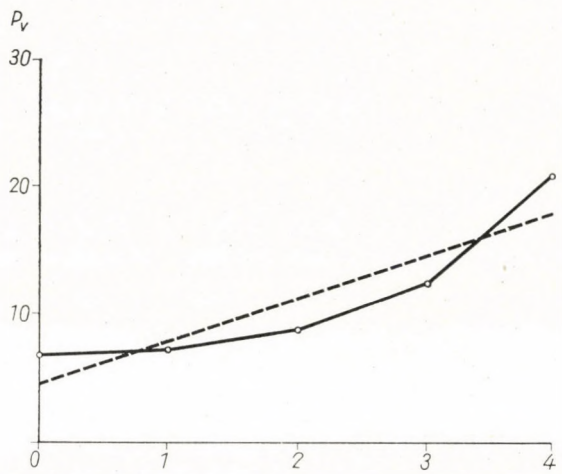

Fig. 6. Regression equation expressing the correlations between severity of oedema (abscissa) and venous pressure in the cases of FARKAS and KORÁNYI

oedema-free condition, then its reduction must amount to 8.7 cm (more precisely, to 8.1 cm only if allowance is made for a minor increase of venous pressure from 6.6 to 7.2). On the other hand, the increase in venous pressure

needs only be half as great, i.e. 3.7 cm (more precisely, 4.4 cm, if allowance is made for a minimal reduction in colloid osmotic pressure from 23.9 to 23.2) to make a moderate oedema severe. The shift from the state of massive to excessive oedema was obviously due to the 8.5 cm increase in venous pressure from 12.5 cm to 21 cm, though colloid osmotic pressure had also increased by

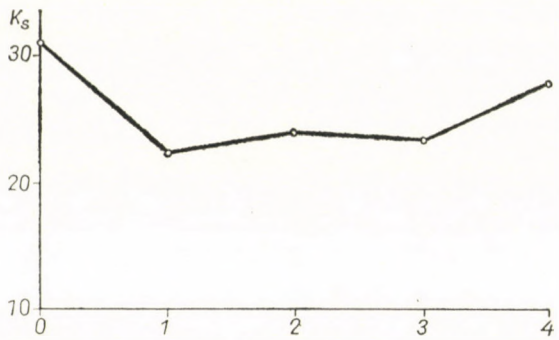

Fig. 7. Regression equation expressing the correlations between severity of oedema (abscissa) and colloid osmotic pressure in the cases of FARKAS and KORÁNYI

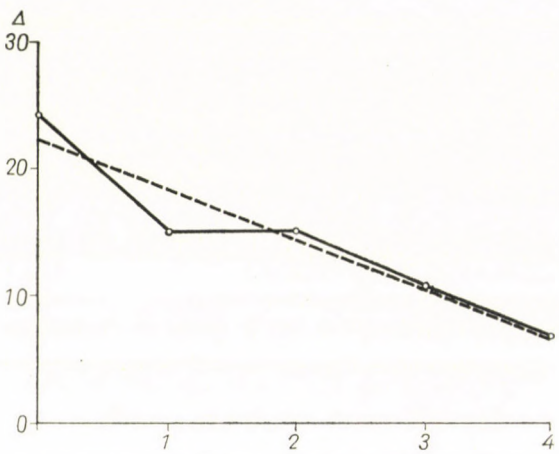

Fig. 8. Regression equation expressing the correlations between severity of oedema (abscissa) and Δ, in the cases of FARKAS and KORÁNYI

4.6 cm, from 23.2 cm to 27.8 cm. This change was caused by a shift of the forces in favour of venous pressure (8.5—4.6 = 3.9 cm); to produce slight oedema in an originally oedema-free state, reduction in colloid osmotic pressure had to attain 8.1 cm: this again demonstrates the ratio of 1 : 2 in the oedematogenic effect of colloid osmotic and venous pressures.

In the cases of KORÁNYI and FARKAS, the correlation coefficient between the degree of oedema and venous pressure was found to be 0.91 ($p < 5\%$) and the regression equation:

$$y = 3.41\,x + 44 \tag{21}$$

The correlation coefficient between the degree of oedema and Δ (the difference between venous and colloid osmotic pressures) was found to be 0.95 ($p<2\%$) and the regression equation:

$$y = -\,3.96\,x + 22.3 \tag{22}$$

The correlation coefficient between the degree of oedema and colloid osmotic pressure was 0.24 only ($p < 70\%$).

These correlations are represented in Figs 6 to 8.

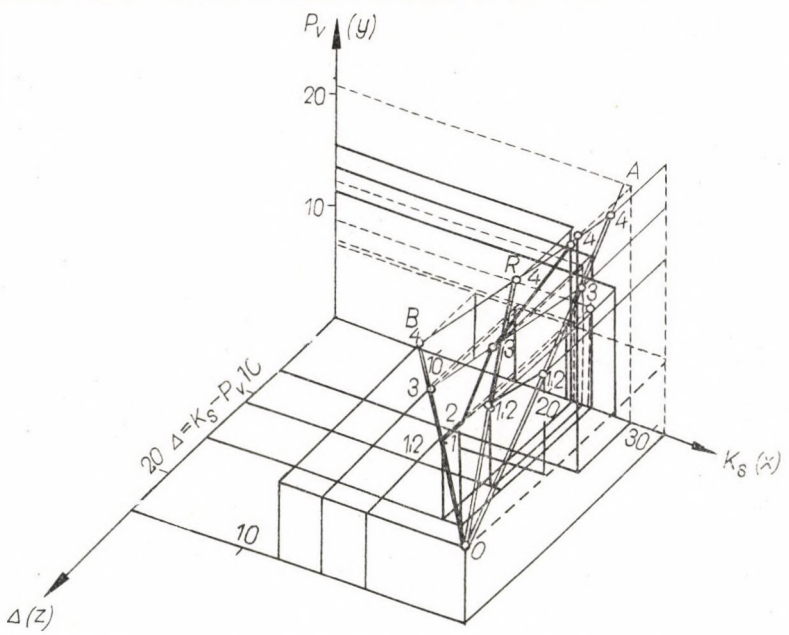

Fig. 9. Spatial straight lines derived from the data obtained by KORÁNYI and FARKAS

For a clearer illustration of these connections, the successively more severe forms of oedema in the KORÁNYI—FARKAS-material may be represented in space as functions of P_v, K_s, and $K_s - P_v$ ($= \Delta$), and the direction of the spatial straight lines obtained in this manner may be compared with that of the theoretically derived straight lines (Fig. 9).

To obtain the 1st theoretical straight line, the K_s values belonging to each of the KORÁNYI—FARKAS Δ-values are computed by leaving P_v unchanged.

A spatial straight line thus obtained reflects the situation where for the increase in oedema a gradual reduction in colloid osmotic pressure would be solely responsible ($O - B$).

To obtain the 3rd theoretical straight line, we have to calculate the P_v-values belonging to the individual Δ-values of KORÁNYI—FARKAS by leaving K_S unchanged. The position of a spatial straight line thus reflects the situation where the increase of oedema would be due solely to a gradual rise in venous pressure ($O - A$).

To obtain the 2nd theoretical spatial line we have to calculate the P_v and K_S values belonging to the individual Δ-values in a manner to having the P_v increase by the same cm H_2O as the K_S is reduced. The position of such a straight line thus reflects the situation where the increase of oedema would be due to a simultaneous reduction in K_S and an elevation in P_v of the same order ($O - R$).

The position of the spatial coordinates indicates that for a slight oedema, in the absence of any previous fluid retention, reduction of K_S is largely responsible, whereas from this stage onward the decisive role is gradually taken over by an increase in P_v.

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MODEL EXPERIMENT FOR THE DEMONSTRATION OF THE EFFECT OF SYSTEMIC PHLEBOHYPERTENSION ON LYMPH FLOW AND OEDEMA PRODUCTION

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It is demonstrable by means of a simple haemo-lymphodynamic model that as a resultant of physical forces, the increased oedematogenic effect of generalized phlebohypertension is closely related to the retroactive spread of increased pressure to the lymphatic system, interfering with the drainage of lymph into the venous system. The differences between "closed" and "cannulated" lymph flow, furthermore the correlations between the disturbances of lymph flow and oedema production are clearly demonstrable in the model.

It has been clearly recognized by STARLING (1909) that systemic phlebohypertension, while leading to an elevation of capillary pressure i.e. of filtration force at the periphery, increasing by this fact the rate of capillary filtration and thus the production of lymph, it inhibits on the other hand the central drainage of lymph into the venous system. STARLING did not, however, regard this inhibition as a significant functional factor.

FÖLDI et al. (1957) have demonstrated in dogs that a sudden elevation of pressure in the superior vena cava is promptly followed by an increase of lymph pressure in the thoracic duct. They have further shown that in central circulatory failure associated with massive oedema the lymph flow from the opened thoracic duct exceeds many times the normal rate; in the intact lymphatic system the velocity of lymph flow is not accelerated.

Later, FÖLDI and PAPP have shown by the bubble-flowmeter technique (1961) and FÖLDI et al. by the thermo-flowmeter technique (1962) that increased lymph flow associated with increased pressure in the inferior vena cava immediately ceases if pressure increases in the superior vena cava. FÖLDI and PAPP (1961) termed the lymph flow in an intact lymphatic system "actual lymph flow" and that demonstrable on thoracic duct cannulation, "cannulated lymph flow". With a normal venous pressure the two values are equal whereas in systemic phlebohypertension "actual lymph flow" is not more than a fraction of "cannulated lymph flow". This is in good agreement with the recent results obtained by WÉGRIA et al. (1963). SERVELLE et al. (1966) inferred from lymphographic findings that in congestive heart failure there is a stasis of lymph in the distended lymphatics.

It seems, therefore, clear that one of the essential factors of cardiac oedema is an increased production of lymph with no increase in the velocity of its flow, or, as we have termed it, a haemodynamic failure of lymph flow.

The reason why it seemed necessary to examine this question is that it has been claimed on the grounds of studies with ^{131}I-albumin that the velocity of lymph flow is increased in cardiac oedema. The method on which the claim has been based is an indirect one. It consists in injecting the labeled albumin subcutaneously and in recording its rate of absorption. Since under normal conditions absorption of the lymph takes place through the lymphatics, changes in the normal rate of absorption are usually identified with those in the rate of lymph flow.

Method

The problem has been studied in a simple model serving as a rough reproduction of haemo-lymphodynamic conditions (Fig. 1). All it had to fulfil

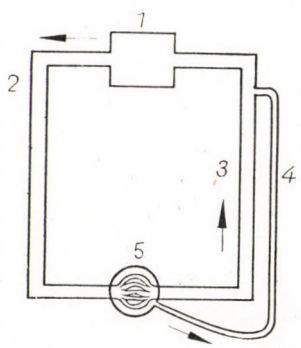

Fig. 1. Scheme of "haemo-lymphodynamic" system. 1. Heart; 2. Arterial system; 3. Venous system; 4. Lymphatic system; 5. Interstitial space with blood and lymph capillaries

was to allow the study of generalized phlebohypertension, further of "actual" and "cannulated" lymph flow. Other complicating factors, such as elasticity and distensibility of the vessels, fluid viscosity, etc. have been neglected.

A constant flow is maintained by a constant pressure in a closed tube system (Fig. 2). In order to reproduce capillary filtration, one portion of the tube runs through a closed elastic cylinder with which it communicates through a small opening (A). A side tube branches from the cylinder at B and joins the main tube at C from which it can be shut off for the outlet of circulating fluid D. The outflow-rate of the fluid at A in a unit of time is obviously related to the pressure prevailing in the system, as well as to the elasticity of the cylinder, and to a certain degree to that of the elastic container.

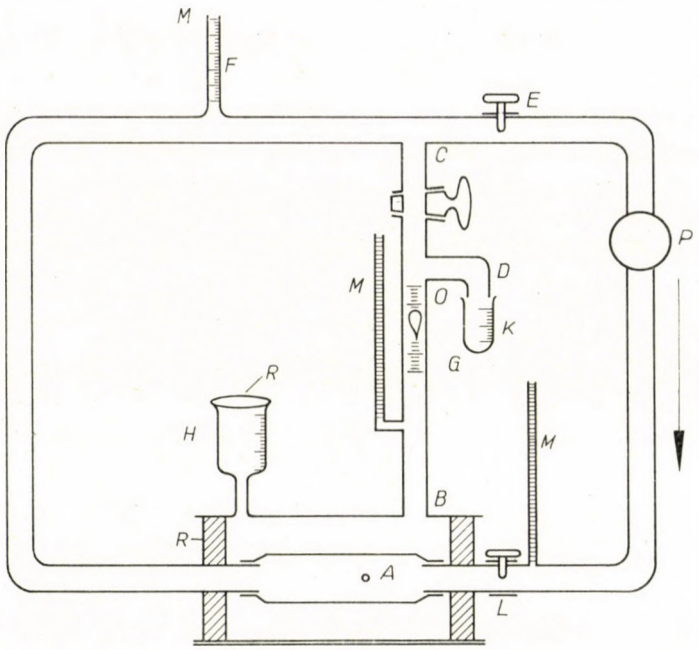

Fig. 2. "Haemo-lymphodynamic" model. P = Pump; M = Manometer; R = Elastic wall. For other abbreviations, see the text

The lumen of the tube has been narrowed at L for the reproduction of peripheral resistance.

We could thus study

a) by rotametric measurement of the flow in a closed system, and

b) by direct measurement of the amount of fluid flowing out at D from the open tube,

in what manner a given restriction of the lumen at E, and an increase of pressure inside the system measured at F, would affect the flow through tube G.

We also studied under the same conditions the pressures in tube G, furthermore the velocity of the rise of the fluid level in container H, this latter serving as a model for the rate of oedema production.

Results

In the case of "closed lymph flow", elevation of "venous pressure" caused a linear reduction of "lymph flow" and an increase in lateral "lymph" pressure (Fig. 3). The correlations between lymph flow and venous pressure are defined by the equation,

$$L = -0.14\, P_v + 78 \qquad (1)$$

or, in a general form

$$L = -aP_v + b \tag{2}$$

where

L represents "lymph flow" expressed in ml per minute,

P_v, "venous pressure" in mm H_2O

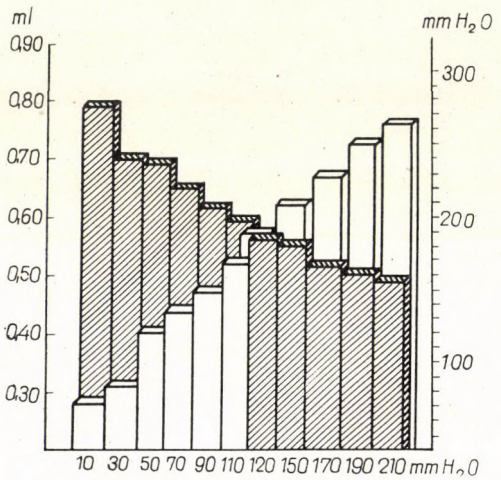

Fig. 3. "Lymph flow" (full columns) and lateral "lymph" pressure (empty columns) in closed "lymph" flow

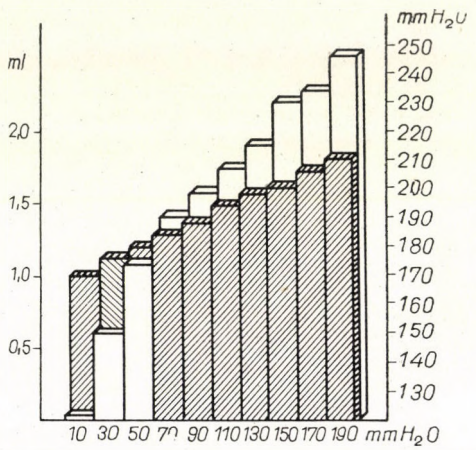

Fig. 4. "Lymph flow" (full columns) and lateral "lymph" pressure (empty columns) in open "lymph flow"

The correlations between lateral pressure of "lymph" and "venous" pressure are defined by the equation,

$$P_1 = 0.85\ P_v + 43 \tag{3}$$

or, in a general form:

$$P_1 = a'\ P_v + b' \tag{4}$$

where P_1 represents "lymph" pressure in H_2O.

In the case of "open lymph flow", elevation of venous pressure caused a linear increase in "lymph flow" and in lateral pressure of "lymph".

The correlations between "lymph flow" and "venous" pressure in case of open flow are described by the following equation:*

$$L = 0.54\ P_v + 70 \tag{5}$$

* If the dimensions are to agree then the gradients of the straight lines may be expressed in $a = \frac{10^{-3}\ \text{ml}}{\text{mm}\ H_2O}$ units.

or

$$L = a'' P_v + b'' \tag{6}$$

The correlation between "lymph" pressure and "venous" pressure is,

$$P_1 = 0.57\ P_v + 119 \tag{7}$$

or

$$P_1 = a''' P_v + b''' \tag{8}$$

When equation (3) is divided by equation (7), the quotient of the gradients of the straight lines is 1.4. This means that in the case of "closed lymph flow" an identical elevation of "venous" pressure entails an increase in lateral "lymph" pressure 1.4 times that in the case of an open flow.

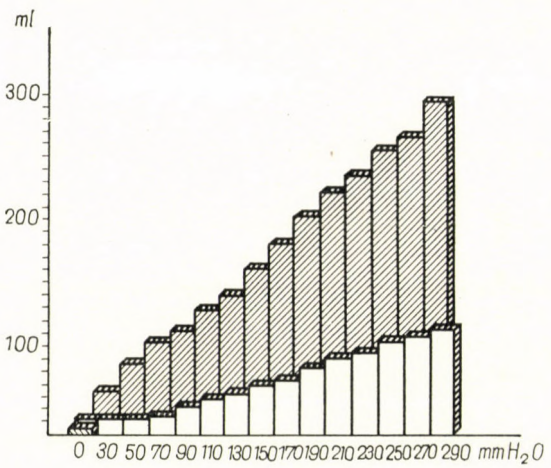

Fig. 5. Rise of "oedema fluid" level upon elevation of "venous" pressure in open (empty columns) and closed "lymph flow" (full columns)

The level of "oedema fluid" showed a linear rise in both open and closed "lymph flow", as described by the equations,

$$O_n = 0.28\ P_v + 33 \tag{9}$$

or

$$O_n = a_1 + P_v + b_1 \tag{10}$$

$$O_z = 0.87\ P_v + 40 \tag{11}$$

or

$$O_z = a_2 P_v + b_2 \tag{12}$$

where

O_n represents the level of "oedema fluid" with open "lymph flow" and O_z the level of "oedema fluid" with closed "lymph flow".

When dividing equation (11) by equation (9), the quotient of the gradients is 3.1. In other words, if in the present haemo-lymphodynamic model the "lymph" is allowed free outflow, on the elevation of "venous" pressure the level of "oedema fluid" rises 3.1 times more slowly than it does if the "lymph" is being returned to the circulation.*

Discussion

The measurements have supported our theoretical deductions and agreed with the results of direct studies of the lymphatic system. On the other hand, they were at variance with the interpretation of the results of studies with ^{131}I-albumin which springs from the misconception that by measuring the rate of ^{131}I-albumin absorption one estimates that of lymph flow. Incidentally, the ^{131}I-albumin method has some additional shortcomings (9).

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* It must be stressed that it is not the absolute value of 3.1 which is significant, only the fact that it is more than 1.

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RADIOZINC UPTAKE BY THE ACUTELY DAMAGED PANCREAS OF RATS

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The uptake of ^{65}Zn glycinate and of ^{65}Zn gluconate by the pancreas, the liver, and the jejunum of albino rats has been studied. In intact animals, the curves for the uptake by the pancreas run high and almost continuous courses from the first to the 7th hour after injection. In laparotomized animals, the pancreas takes up much less, the liver more, and the jenunum about as much of the injected ^{65}Zn as it does in intact rats. In animals with desoxycholate-induced pancreatitis, ^{65}Zn uptake by the pancreas is slight even in relation to that in laparotomized rats, less so the uptake by the jejunum, but that by the liver remains unchanged. The decrease in uptake is probably due to decreased blood flow through the inflamed pancreas.

The pancreas, the liver, and the intestines of the normal laboratory animal take up much ^{65}Zn [10, 14]. Uptake by the pancreas and the liver reaches its peak 2 hours after intravenous injection in mice, and 6 to 8 hours in dogs [7, 14]. In fact, the high affinity of the pancreas for ^{65}Zn has prompted many attempts to use this isotope in making the organ visible (cf. [10]).

Data are scarce concerning the uptake of ^{65}Zn by the abdominal organs under pathological conditions. LOWRY et al. [5] found that the pancreas of rats with alloxan diabetes took up much less ^{65}Zn in the first 24 hours after injection than did the pancreas of control animals.

In the present work we have investigated the effect of acute pancreatic injury on the ^{65}Zn uptake by the pancreas, the liver, and the jejunum.

Material and methods

Random-bred female Wistar-strain rats weighing 180 to 250 g were placed in six treatment groups. After treatment in one of the six ways detailed below and designated A through F, all the animals were anaesthetized with ether and given an injection of ^{65}Zn glycinate or ^{65}Zn gluconate in the long saphenous vein. Each rat received 0.5 ml of a solution containing 10 μg of zinc (activity 8.5 to 9.5 μC; molar ratio of Zn to glycinate and of Zn to gluconate 1 : 2). With the exception of those in group A, all animals were decapitated 2 hours after injection. Blocks of 100 to 300 mg were at once removed from the pancreas, the liver, and the jejunum, carefully cleansed, and weighted with a torsion balance. Activities were measured in a well-type scintillation counter, and the differential absorption ratios were calculated:

$$\frac{\text{cpm/g organ weight}}{\text{administered cpm/g body weight}}$$

For histology, small blocks of pancreas and liver were fixed in formalin and stained with haematoxylin-eosin.

Statistical significance was determined by Student's "t" test.

Treatment groups

A) After a 16-hour fast, ^{65}Zn was injected and the animals were decapitated 1, 2, 3, 5, 7 and 24 hours later.

B) After a 16-hour fast, laparotomy was performed under ether anaesthesia. Still in the fasting state, the animals were given ^{65}Zn on the next day.

C) Rats were treated as in group B, but after opening the abdominal wall the duodenum was pierced and a cannula was inserted into the bile duct through which 0.6 ml of a 1.5 per cent sodium desoxycholate solution (pH 7.4) was injected slowly into the pancreas under compression of the common duct for 2 minutes [2].

D) To serve as controls for groups E and F, rats were injected intraperitoneally on two, consecutive days with a 1 ml/100 g dose of physiological saline and given ^{65}Zn on the third day, after a 16-hour fast.

E) Rats were injected intraperitoneally on two consecutive days with a 25 mg/kg dose of d.l.-ethionine in physiological saline and given ^{65}Zn on the third day, after a 16-hour fast.

F) Under ether anaesthesia, the rats received into the saphenous vein a single injection of 4 mg per 100 g of alloxan in 0.25 ml of physiological saline and were given ^{65}Zn three days later, after a 16-hour fast.

The rats in groups D, E, and F were treated simultaneously.

Results

Control group A. The uptake by the pancreas of ^{65}Zn glycinate and of ^{65}Zn gluconate ran a high and almost continuous course from the first to the 7th hour after injection. Twenty-four hours later it was found to have fallen to between 1/3th to 1/4th of the peak value (Fig. 1).

Laparotomized group B. The pancreas took up much less, the liver more, and the jejunum about as much of ^{65}Zn as they did in the intact control rats (Table I).

Pancreatitic group C. In these animals pancreatic ^{65}Zn uptake was slight even in relation to that in the laparotomized rats, less so the uptake by the jejunum, but that by the liver remained unchanged (Table I). ^{65}Zn uptake showed the same decrease whether calculated for dry or wet weight.

Histology revealed inflamed necrotic areas of variable size in about half the number of blocks of pancreas removed from the pancreatitic rats in group C. In one quarter, mild inflammatory infiltrations were seen, without necroses. In the rest, no histological changes were detected.

Ethionine-treated group E. The pancreas took up as much ^{65}Zn glycinate in the treated animals as in the untreated control rats. The uptake by the jejunum was higher and that by the liver lower than in the controls. This treatment induced vacuolar degeneration and, occasionally, interstitial infiltration and cellular dissociation in the pancreas and the liver (Table II).

Alloxan-treated group F. The uptake of ^{65}Zn glycinate by each of the three organs studied was the same in the treated as in the control animals despite a decrease in number and diameter of the islets of Langerhans (Table II).

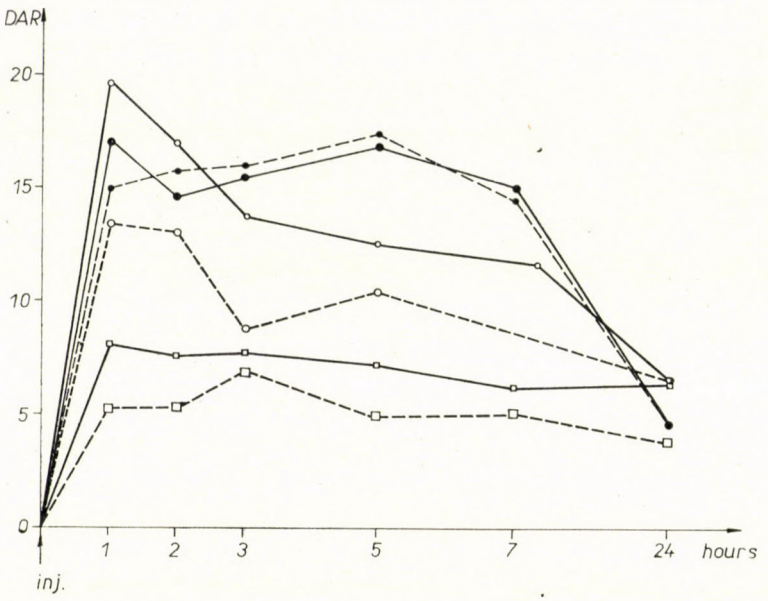

Fig. 1. Time course of ^{65}Zn uptake of pancreas (●), liver (○), and jejunum (□). ^{65}Zn glycinate -------; ^{65}Zn gluconate ———. Each point is the mean of 4 values

Table I

^{65}Zn uptake in experimental pancreatitis

	Treatment groups	Number of rats	pancreas*	liver*	jejunum*
^{65}Zn glycinate	A Control**	9	15.5 ± 1.2	13.5 ± 0.6	6.0 ± 0.7
	B Laparotomy	10	7.6 ± 0.8	16.2 ± 0.7	5.7 ± 0.4
	C Pancreatitis	6	2.1 ± 0.3	16.2 ± 0.9	4.0 ± 0.4
	Significance		A—B $p<0.001$	A—B $p<0.05$	
			B—C $p<0.001$		B—C $p<0.02$
^{65}Zn gluconate	A Control**	9	14.6 ± 0.9	14.8 ± 1.0	7.2 ± 0.5
	B Laparotomy	13	11.5 ± 0.9	21.9 ± 0.9	6.8 ± 0.6
	C Pancreatitis	16	4.4 ± 0.5	20.4 ± 0.8	4.9 ± 0.4
	Significance		A—B $p<0.05$	A—B $p<0.001$	
			B—C $p<0.01$		B—C $p<0.01$

* $\frac{\text{cpm/g organ weight}}{\text{administered cpm/g body weight}}$, mean ± S. E.

** 2 hours after injection

Table II

^{65}Zn glycinate uptake after treatment with ethionine and alloxan

Treatment groups	Number of rats	pancreas*	liver*	jejunum*
D Control	11	11.6±0.9	14.1±0.9	6.0±0.4
E Ethionine	5	10.5±1.2	7.1±0.5	8.5±0.2
F Alloxan	10	11.4±1.2	12.6±0.6	6.5±0.4
Significance			D−E $p<0.001$	D−E $p<0.01$

* $\frac{\text{cpm/g organ weight}}{\text{administered cpm/g body weight}}$

Discussion

Intravenously injected ^{65}Zn is rapidly bound by the plasma proteins and the blood cells, is not dialysable [4, 11, 13], and the blood is quickly cleared of it [3]. OKUNEWICZ et al. [8] put forward the hypothesis that ^{65}Zn is transferred from plasma to tissue by direct physicochemical exchange. Experiments *in vitro* revealed that substances inhibiting metabolism leave the uptake of ^{65}Zn by the liver unaffected; its passage through the cell membrane is probably a passive one, ant it becomes bound inside the cell [12].

In the pancreas the ^{65}Zn is taken up by the acinar cells and the islets of Langerhans, which latter account for about 3 per cent of the pancreatic mass. In the acinar cells ^{65}Zn concentration reaches its peak in the first 7 hours and is at a much lower level 24 hours later. In the islets of Langerhans it remains virtually unchanged in the first 48 hours after injection [6]. In the first 7 hours uptake by the acinar cells predominates. This easily explains why in our experiments alloxan failed to influence ^{65}Zn uptake by the end of the second hour. As has already been mentioned, LOWRY et al. [5] have found that the pancreas of rats with alloxan diabetes takes up less ^{65}Zn in the first 24 hours after injection than does the pancreas of control animals; it seems probable that at this time the greater part of the total activity of the pancreas originates from the isotope retained by the islets of Langerhans.

Treatment with ethionine, which interferes with pancreatic protein synthesis did not affect early ^{65}Zn uptake by the gland [1]. This agrees well with the mentioned hypothesis of OKUNEWICZ et al. [8] that uptake rests on direct physico-chemical exchange.

In the rats with sodium desoxycholate-induced pancreatitis, ^{65}Zn uptake by pancreas and jejunum was decreased. Obviously, the substantial decrease in pancreatic uptake could not have been caused by oedema since the decrease was found to be the same whether calculated for dry or for wet weight, and

it seems likely that it was a consequence of the reduced blood flow of the inflamed pancreas [9]. The decrease in ^{65}Zn uptake by the jejunum may have been the consequence of a reduction in the amount of blood flowing through the splanchnic vascular areas following a shifting of circulating blood brought about by peritoneal excitation in laparotomized and by shock in the pancreatitic animals.

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EFFECTS OF HYPOPHYSECTOMY AND OF ACTH ON THE CHANGES INDUCED BY HEXADIMETHRINE BROMIDE

By

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Hexadimethrine bromide was found to induce in hypophysectomized rats a sharp fall in rectal temperature and to lead to death in a few hours. Necrosis of the inner layers of the adrenal cortex and of the ascending limbs of HENLE's loops is less severe than in the controls, and nephrocalcinosis remains absent, but the necroses in the zona glomerulosa are not affected by hypophysectomy. In hexadimethrine bromide treated animals 24 hours after hypophysectomy ACTH-pretreatment decreases the fall of rectal temperature, prolongs the survival but increases the severity of adrenocortical, hepatic, and renal necrosis. Animals treated with hexadimethrine bromide one month after hypophysectomy are less responsive to ACTH; in these cases ACTH fails to inhibit the fall of rectal temperature and to prolong survival, but also increases the severity of necroses. It is concluded that hypophysectomy affects the consequences of hexadimethrine bromide treatment by reducing the resistance of rats while protecting them from tissue damage.

Hexadimethrine bromide administered to rats induces patchy infarction of the anterior lobe of the pituitary, of the inner layers of the adrenal cortex and of the liver and extensive necrosis of the zona glomerulosa of the adrenal cortex. It also affects the kidney by causing necrosis and calcification of HENLE's loops (SELYE et al., 1963, CARROLL et al., 1964, KOVÁCS et al., 1964a, 1966a, NICHOLS, 1966, KOVÁCS and SZIJJ, in press). Since hypophysectomy impairs the resistance of rats to various injuries and modifies experimental damage to the adrenals and to the kidneys, it seemed interesting to examine whether the damage would be modified by previous hypophysectomy or by ACTH administration. The present report deals with these studies and their results.

Method

We used female albino rats of identical strain and of 160 to 220 g body-weight, keeping them on a standard diet. Six groups were set up. The first comprised untreated controls. The animals of the second group received 5 mg hexadimethrine bromide (Polybrene, Abbott) into a tail vein. The animals of groups 3 to 6 were hypophysectomized under ether anaesthesia from the parapharyngeal approach. The animals of the third group received 5 mg hexadimethrine bromide into the tail vein 24 hrs after hypophysectomy on a single occasion without any other treatment. The animals of the fourth group received ACTH in subcutaneous doses of 5 IU, the first being given immediately after hypophysectomy, the second and third 12 and 24 hrs later. Together with the last injection 5 mg hexadimethrine bromide were injected intravenously. The animals of the fifth group were given a single intravenous dose of 5 mg hexadimethrine

bromide one month after hypophysectomy without any other treatment. In the sixth group 5 IU ACTH were given subcutaneously one month after hypophysectomy on four subsequent days. These animals received 5 mg hexadimethrine bromide together with the last injection.

Rectal temperature was measured with a mercury thermometer at determined intervals before and after the administration of hexadimethrine bromide. The time of death was recorded and mean survival assessed 1, 2, 3, 4, 6, 8, 12, 24, 36, and 48 hrs after the injection. The surviving animals were killed by ether inhalation 48 hrs after the hexadimethrine bromide administration. After death the sellar region was examined so as to ascertain that hypophysectomy had been complete. Animals with residual pituitary tissue were not evaluated. The organs removed at autopsy were fixed in 4 per cent formaldehyde and embedded in paraffin. Sections 4 to 8 μ thick were stained with hematoxylin-eosin. The extent of necrosis found in the organs was graded 1, 2, 3, and 0; 1 signifying small, 2 medium, 3 extensive necrosis and 0 no microscopic evidence of necrosis. The individual organs of the animals belonging to the same group were graded separately, and the sums of the figures were divided by the number of animals. The figure thus obtained, termed necrosis index, was the result of qualitative assessment, nevertheless it reflects the extent and incidence of the changes with reasonable accuracy and lends itself for this reason to a comparative evaluation of the findings between the individual groups. A nephrocalcinosis index was computed in the same manner. The prerequisite for evaluating changes in the adrenals and in the liver was a survival of at least six hrs, and those in the kidneys of at least 24 hrs, since signs of necrosis are not manifest before that time.

Results

Death rate and mean survival time are presented in Table I. In the group of non-hypophysectomized animals not more than 4 out of 22 died within 48 hrs after the administration of hexadimethrine bromide. Tolerance to the drug was greatly affected by hypophysectomy, most animals died by the end of 48 hrs. Resistance seemed to have been lowest in the rats hypophysectomized 24 hrs previously, as none of these animals was alive 24 hrs after the hexadimethrine bromide administration. Survival of the animals injected with hexadimethrine bromide one month after hypophysectomy was considerably shorter than of non-hypophysectomized ones, but considerably longer than of those which had been hypophysectomized 24 hrs prior to the injection of hexadimethrine bromide. ACTH was found considerably to prolong survival in animals injected with hexadimethrine bromide 24 hrs after hypophysectomy; some of them were still alive 48 hrs after the injection. In opposition to this, ACTH failed to affect survival in the animals injected with hexadimethrine bromide one month after hypophysectomy. Though in the first six hours less animals died in the group which had been given ACTH together with hexadimethrine bromide than in that which had received hexadimethrine bromide alone, later this difference was no longer demonstrable and mean survival was almost the same in both groups.

Table II shows the rectal temperatures measured at different times. While temperature of the controls was practically unchanged throughout, administration of hexadimethrine bromide to intact animals was followed by a moderate fall of temperature. In the hypophysectomized animals the rectal temperatures had been lower prior to the administration of hexadimethrine bromide and from the injection of hexadimethrine bromide onward tempera-

Table I

Group	No. of animals	Died within			Average survival hours	
		6	24	48		
		hours			of all rats	of dead rats
Non-hypophysectomized, untreated	10	0	0	0	48	—
Non-hypophysectomized + HB	22	0	1	4	46	36
Hypophysectomy one day earlier + HB	13	10	13	13	4	4
Hypophysectomy one day earlier + ACTH + HB	13	1	5	7	34	22
Hypophysectomy one month earlier + HB	18	6	11	12	24	12
Hypophysectomy one month earlier + ACTH + HB	15	1	9	10	27	16

HB = hexadimethrine bromide

ture decreased rapidly and most animals died within two days. Temperature of hypophysectomized animals injected with ACTH was higher initially than of those which received no ACTH. Hypothermia due to hexadimethrine bromide injected 24 hrs after hypophysectomy was considerably slighter, or occasionally even absent, in the animals pretreated with ACTH. In contrast, ACTH had no protective influence in those animals which had been treated with hexadimethrine bromide one month after hypophysectomy, as reflected by a considerable fall of temperature.

Table III sums up the incidence of necrotic changes and the necrosis-index values in the individual organs. Hexadimethrine bromide caused anterior pituitary necrosis in 10, infarcts in the inner layers of the adrenal cortex in 12, infarcts in the liver in 9 out of 22 non-hypophysectomized rats. Necrosis of the zona glomerulosa and of HENLE's loops was demonstrable in all, nephrocalcinosis in 9 of the animals. Incidence and extent of the necrotic changes affecting the individual organs largely corresponded to earlier findings (CARROLL et al., 1964; KOVÁCS et al., 1964a, 1966a; KOVÁCS and SZIJJ, in press). All the animals treated with hexadimethrine bromide 24 hrs after hypophysectomy died within 24 hrs after the injection and no more than 3 survived the first six hrs. Since development of demonstrable adrenocortical and hepatic necrosis takes about six hrs and that of renal necrosis 24 hrs, we had no opportunity to study necrotic lesions of the adrenal cortex and of the liver in more than 3 animals and those of the kidneys in none at all. In these 3 animals infarction of

Table II

Group	No. of animals	No. of animals dead within 48 hrs	Rectal	
			0	1
Non-hypophysectomized, untreated	10	0	38.5 ± 0.2*	38.3 ± 0.2
Non-hypophysectomized + HB	10	0	38.6 ± 0.2	36.5 ± 0.3
Hypophysectomy one day earlier + HB	13	13	35.4 ± 0.5	35.6 ± 0.5
Hypophysectomy one day earlier + ACTH + HB	13	7	38.6 ± 0.4	37.0 ± 0.2
Hypophysectomy one month earlier + HB	18	12	37.4 ± 0.1	34.2 ± 0.4
Hypophysectomy one month earlier + ACTH + HB	15	10	38.0 ± 0.1	35.4 ± 0.3

HB = hexadimethrine bromide
* = standard error of the mean
** = less than three rats alive

the inner adrenocortical layers was found in one instance and necrosis of the zona glomerulosa in three. Hepatic necrosis was not observed. It is, however, clear that the findings derived from a total of three animals give no true estimate of the incidence and extent of necrotic lesions affecting the individual organs. In the ACTH-pretreated animals the necrosis induced by hexadimethrine bromide in the adrenal cortex, in the liver, and in HENLE's loops when administered 24 hrs after hypophysectomy showed the same incidence and extent as in non-hypophysectomized animals treated with hexadimethrine bromide. Nephrocalcinosis affecting the cortico-medullar junctions also developed. In the group where a month had elapsed between hypophysectomy and hexadimethrine bromide administration the atrophied deeper layers of the adrenal cortex contained occasional minute infarcts only. Necrosis of the liver was absent in this group and that of HENLE's loops was slight. In 2 out of 7 animals whose organs had been studied microscopically, there was no evidence of renal necrosis and in the remaining 5, necrosis was of patchy character and not extensive. Nephrocalcinosis was absent throughout. In the group where administration of hexadimethrine bromide four weeks after hypophysectomy had been combined with ACTH-pretreatment, infarcts of the deeper adrenocortical

temperature at hour

2	3	4	6	8	12	24	36	48
38.3 ± 0.3	38.2 ± 0.3	38.1 ± 0.3	37.6 ± 0.2	38.1 ± 0.2	38.1 ± 0.2	37.6 ± 0.1	38.3 ± 0.2	38.6 ± 0.1
36.2 ± 0.3	36.5 ± 0.4	36.9 ± 0.5	35.8 ± 0.3	36.7 ± 0.4	36.7 ± 0.3	35.9 ± 0.3	36.8 ± 0.3	35.1 ± 0.4
34.8 ± 0.6	33.8 ± 0.7	32.8 ± 1.0	31.1 ± 0.8	32.0**	—	—	—	—
36.9 ± 0.2	37.2 ± 0.2	37.3 ± 0.3	36.9 ± 0.4	37.8 ± 0.3	38.5 ± 0.2	37.1 ± 0.1	38.7 ± 0.3	36.2 ± 0.7
33.4 ± 0.6	33.3 ± 0.6	33.3 ± 0.7	32.8 ± 0.8	32.8 ± 0.7	33.5 ± 0.1	33.5 ± 0.7	33.8 ± 0.5	33.4 ± 0.5
34.2 ± 0.4	34.3 ± 0.4	34.0 ± 0.4	33.2 ± 0.4	34.4 ± 0.4	35.8 ± 0.4	34.3 ± 0.3	36.1 ± 0.3	31.9 ± 0.8

layers were more frequent, though definitely not more extensive than in the other groups, but necrosis of the zona glomerulosa was demonstrable throughout. Infarcts were frequent in the liver. Necrosis of HENLE's loops was also demonstrable in those animals which had survived 24 hrs, but nephrocalcinosis was absent.

The microscopic changes demonstrable in the intact animals treated with hexadimethrine bromide corresponded to those described earlier (CARROLL et al., 1964; KOVÁCS et al., 1964a, 1966a; KOVÁCS and SZIJJ, in press). In the anterior pituitary, the inner layers of the adrenal cortex and in liver they were characteristic infarcts. The zona glomerulosa showed extensive necrosis; in a number of animals it was narrow or had disappeared, subcapsularly zona fasciculata cells were recognizable. Renal damage was found to affect the cortico-medullar boundary. The tubular epithelium lining the ascending limbs of HENLE's loops was necrosed and there was nephrocalcinosis in a number of animals.

The adrenal cortex of the animals which had been hypophysectomized one month before hexadimethrine bromide treatment, displayed definite atrophy affecting prevalently the inner layers. The zona glomerulosa appeared broader and was sharply demarcated from the zona fasciculata. In the ACTH-pretreated animals broadening of the atrophic cortex and enlargement of the cells were demonstrable. In the animals which had been hypophys-

Table III

Group	No. of animals	No. of animals surviving			Adenohypophysis	
		6	24	48		
		hours			number	index
Non-hypophysectomized, untreated	10	10	10	10	*0/10**	0.0
Non-hypophysectomized + HB	22	22	21	18	10/22	0.7
Hypophysectomy one day earlier + HB	13	3	0	0	—	—
Hypophysectomy one day earlier + ACTH + HB	13	12	8	6	—	—
Hypophysectomy one month earlier + HB	18	12	7	6	—	—
Hypophysectomy one month earlier + ACTH + HB	15	14	6	5	—	—

HB = hexadimethrine bromide
* = number of necroses
** = number of microscopically evaluated animals

ectomized 24 hrs prior to hexadimethrine bromide, cortical atrophy was not evident. Treatment with ACTH induced cortical hyperplasia. In the adrenals of those hypophysectomized animals in which hexadimethrine bromide had been lethal within the first hours, extensive hyperaemia, capillary distension, and haemorrhages were found in a number of cases. The deeper layers of the zona fasciculata and the zona reticularis were particularly affected. These animals do not figure in Table III having died before the possible necrosis had become manifest. In those animals which survived the first six hours after hexadimethrine bromide administration the infarcts in the inner layers of the adrenal cortex, though being generally small, did not differ in character from those found in the animals which had not been hypophysectomized. Necrosis of the zona glomerulosa and the renal lesions had the same microscopic appearance as those caused by hexadimethrine bromide in the non-hypophysectomized animals. The histological changes are presented in Figs 1 to 10.

Discussion

The present results clearly show that hypophysectomy increases the vulnerability of rats to hexadimethrine bromide. Administration of the substance is followed by a sharp fall of rectal temperature and the majority of the animals die within a few hours particularly if the interval between hypophysec-

Zona fasc. and zona retic.		Liver		Zona glomerulosa		Henle's loops		Nephrocalcinosis	
N e c r o s i s									
number	index	number	index	number	index	number	index	number	index
0/10	0.0	0/10	0.0	0/10	0.0	0/10	0.0	0/10	0.0
12/22	1.2	9/22	0.9	22/22	2.5	21/21	2.2	9/21	0.8
1/3	0.7	0/3	0.0	3/3	2.0	—	—	—	—
7/12	0.8	9/12	1.2	12/12	2.3	8/8	2.3	5/8	0.9
4/12	0.4	0/12	0.0	12/12	2.2	5/7	0.9	0/7	0.0
9/14	0.9	12/14	1.6	14/14	2.3	6/6	1.7	0/6	0.0

tomy and hexadimethrine bromide treatment was as short as 24 hrs. This observation is in no way surprising, hypophysectomy being known to affect the resistance of animals to other stressors as well (SELYE, 1947). What impressed us

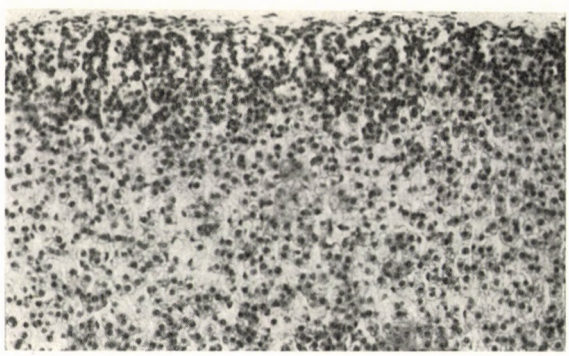

Fig. 1. Adrenal gland of normal control rat. The zona glomerulosa and zona fasciculata are clearly seen. Haematoxylin—eosin, ×176

was the different tolerance of the animals to hexadimethrine bromide given 24 hrs or four weeks after hypophysectomy. Tolerance to hexadimethrine bromide administered 24 hrs after hypophysectomy was poor, probably in consequence of acute corticosteroid depletion, and the animals died without exception in the first hours. The animals which received the drug one month after hypophysectomy seem to have acquired a slight capacity of adaptation to the

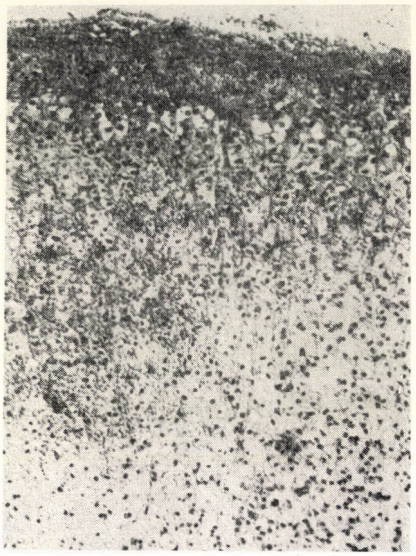

Fig. 2. Adrenal gland of non-hypophysectomized rat 24 hrs after administration of hexadimethrine bromide. Complete destruction of the zona glomerulosa. Haemorrhagic necrosis in the zona fasciculata. Haematoxylin—eosin, ×176

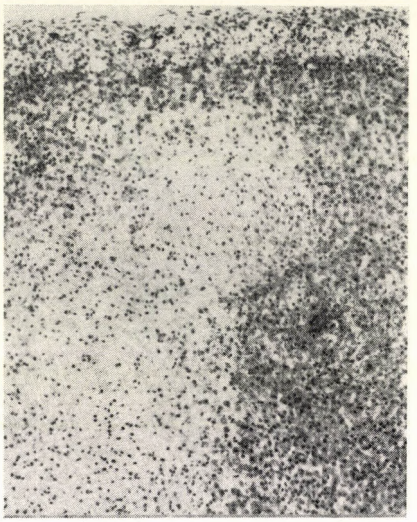

Fig. 3. Adrenal gland of non-hypophysectomized rat 24 hrs after administration of hexadimethrine bromide. Necrosis of zona glomerulosa. Focal infarction in the zona fasciculata. Haematoxylin—eosin, ×110

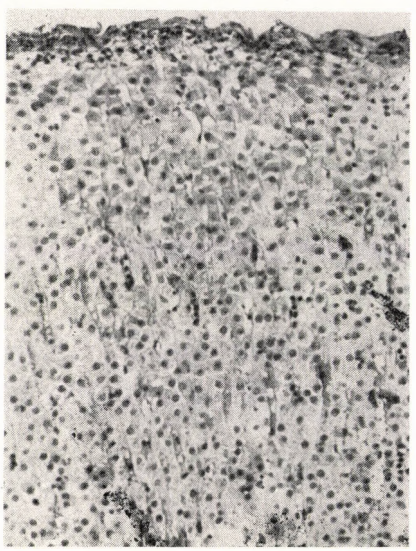

Fig. 4. Adrenal gland of non-hypophysectomized rat 48 hrs after administration of hexadimethrine bromide. The necrotic zona glomerulosa has practically disappeared. Haematoxylin—eosin, ×176

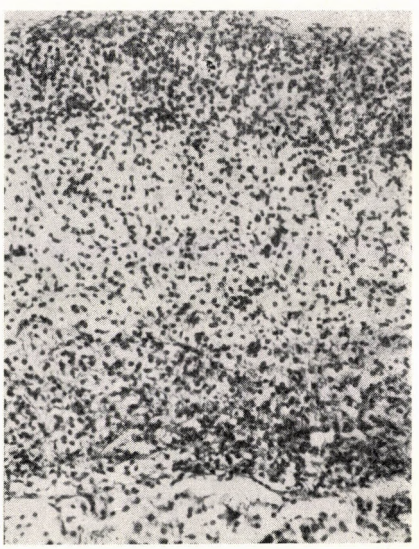

Fig. 5. Adrenal gland of untreated rat one month after hypophysectomy. The zona glomerulosa appears to be broader, whereas the zona fasciculata and zona reticularis are definitely narrow. Haematoxylin—eosin, ×176

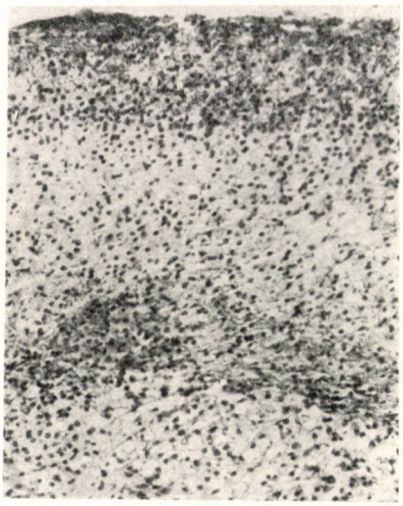

Fig. 6. Adrenal gland of rat 48 hrs after administration of hexadimethrine bromide. Hypophysectomy one month earlier. Definite adrenocortical atrophy. Complete destruction of the broad zona glomerulosa. The zona fasciculata and zona reticularis are narrower but free from necrosis. Haematoxylin—eosin, ×176

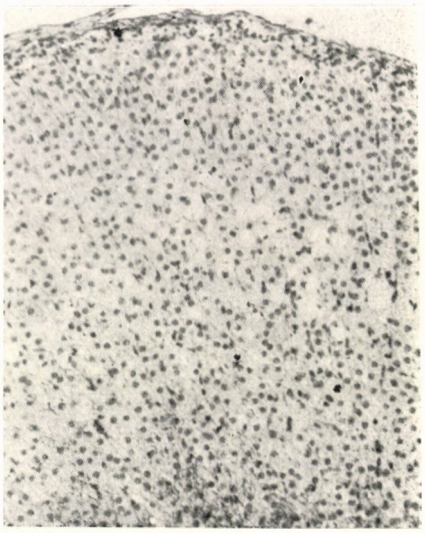

Fig. 7. Adrenal gland of rat 48 hrs after administration of hexadimethrine bromide. Hypophysectomy one month earlier. ACTH-pretreatment. Complete destruction of the zona glomerulosa. The zona fasciculata shows no evidence of necrosis. Haematoxylin—eosin, ×176

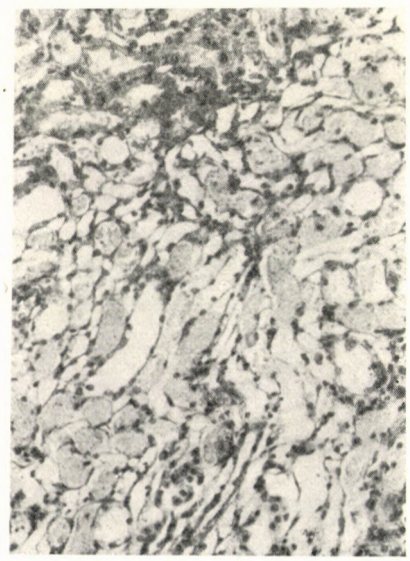

Fig. 8. Kidney of non-hypophysectomized rat 48 hrs after administration of hexadimethrine bromide. Extensive necrosis of the ascending limbs of HENLE's loops. Haematoxylin—eosin, ×176

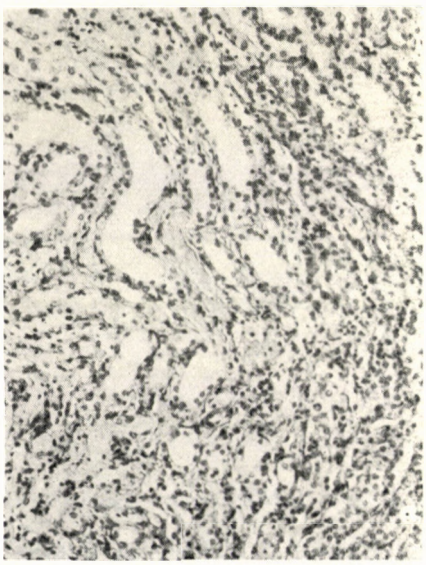

Fig. 9. Kidney of rat 48 hrs after administration of hexadimethrine bromide. Hypophysectomy one month earlier. Necrosis of HENLE's loops is not extensive. Haematoxylin—eosin, ×176

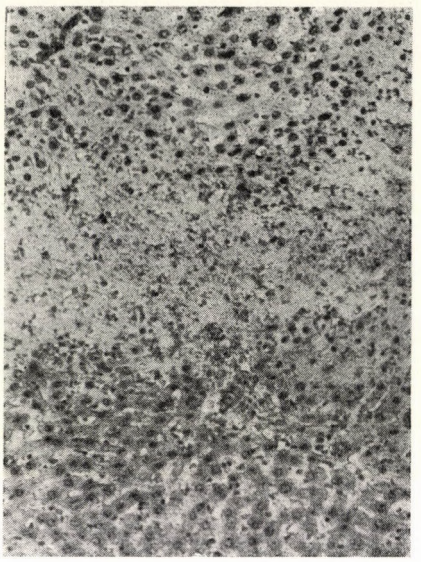

Fig. 10. Liver of rat 48 hrs after administration of hexadimethrine bromide. Hypophysectomy one month earlier. Pretreatment with ACTH. Characteristic patchy infarction. Haematoxylin—eosin, ×176

corticoid deficiency, probably by a reduction of their demands in corticoids which results in a certain resistance. Another distinctive feature between the two groups treated with hexadimethrine bromide 24 hrs and four weeks respectively after hypophysectomy was the response to ACTH. Treatment 24 hrs after hypophysectomy with ACTH was found to prolong survival and to counteract the fall of rectal temperature. In the animals treated four weeks after hypophysectomy the effect of ACTH was less marked. It hardly altered survival and failed to avert the fall of rectal temperature. It did, however, have an influence on the adrenal cortex even in these cases by leading to definite broadening of the atrophied inner layers. It also increased the frequency of necrosis in the individual organs. Responsiveness of the adrenal cortex to the enhancing influence of ACTH was reduced in animals which had been hypophysectomized four weeks earlier. This is also supported by the observation of DEAR and GUILLEMIN (1960) that ACTH fails to elicit a similar elevation of the plasma corticosterone level in animals hypophysectomized several weeks before as in those which were hypophysectomized a few hours earlier. Poor adrenocortical response to ACTH in rats hypophysectomized several weeks previously has been observed also by us earlier (DÁVID et al., 1961).

Hypophysectomy affected not only rectal temperature and survival, but also the frequency and extent of necroses in the individual organs. Though the number of survivors beyond the first hours of hexadimethrine bromide effect was too small to make reliable evaluation possible, the findings clearly showed

that hypophysectomy protects the tissues against the effects of hexadimethrine bromide, whereas ACTH makes them again susceptible to necrosis, with the sole exception of the zona glomerulosa where the necrosis caused by hexadimethrine bromide was not affected by hypophysectomy or ACTH.

The significant influence of hypophysectomy or of ACTH on necrotic lesions has been pointed out by several investigators. TONUTTI (1953) found hypophysectomy to protect guinea pigs from adrenal necrosis induced by diphtheria toxin unless ACTH treatment was applied simultaneously in which case hypophysectomy had no protective effect at all. Dimethylbenzanthracene fails to produce adrenocortical necrosis in rats hypophysectomized a few weeks earlier (HUGGINS and MORII, 1961; MORII and HUGGINS, 1962; HUGGINS and SUGIYAMA, 1965). ACTH was found to enhance the induction of adrenal necrosis by thrombin (MARGARETTEN et al., 1965), by dimethylbenzanthracene (MORII and HUGGINS, 1962), and by thorium dextrine (GABBIANI et al., 1965) in intact rats. In opposition to the present results, TUCHWEBER et al. (1963) found hypophysectomy to have an aggravating, and ACTH a protective, influence on adrenocortical necrosis induced by hexadimethrine bromide. Studying the effects of hexadimethrine bromide on the adrenal cortex in rats hypophysectomized three to six weeks earlier, HUGGINS and SUGIYAMA (1965) found that adrenocortical necrosis developed in these animals also. These results are, however, by no means comparable to ours since these authors had used massive doses — 6 mg per 100 g body-weight — which invariably proved lethal to our animals in a few minutes regardless of previous hypophysectomy. Our results are in agreement with those of NICHOLS (1966) who found that hexadimethrine bromide was poorly tolerated by rats hypophysectomized one week earlier and the dose of 1.6 mg per 100 g was lethal for all the animals in two to twelve hours. Hyperaemia and capillary distension were seen, but adrenocortical necrosis was absent.

Hypophysectomy is known to modify the development of renal changes. It was found to inhibit compensatory hypertrophy of the contralateral kidney after unilateral nephrectomy (ASTARABADI, 1963), to reduce or to avert entirely the renal changes caused by parathormone, dihydrotachysterol, chromium chloride and NaH_2PO_4 (SELYE, 1958; SELYE et al., 1962a, b). According to FARNSWORTH (1959) aminonucleoside nephrosis fails to develop in hypophysectomized rats. We, too, found that hypophysectomy reduced the severity of pituitrin-induced focal necrosis of the renal cortex in oestrogen-pretreated rats (KOVÁCS et al., 1964b). The present results are in agreement with those of TUCHWEBER et al. (1963) according to which hexadimethrine bromide administered to rats immediately after hypophysectomy does not cause nephrocalcinosis unless preliminary treatment with ACTH has been applied.

The mechanism underlying the protective effect of hypophysectomy is not clear. Our earlier studies (CARROLL et al., 1964; KOVÁCS et al., 1966a; KOVÁCS

and SZIJJ, in press) suggest that the necroses in the adrenal cortex and in the liver are due to ischaemia arising as a consequence of some, still unexplained, disorder of regional blood flow. We would have to clarify whether the primary cause of ischaemia was capillary damage, stasis, vasospasm or thrombosis (KOVÁCS et al., 1966b; KOVÁCS, 1967) in order to offer any explanation accounting for the protective action of hypophysectomy. We have shown earlier that administration of massive doses of posterior pituitary hormone to oestrogen-pretreated rats leads to patchy necrosis of the renal cortex by causing reno-vascular spasm. Though hypophysectomy failed to inhibit the vasospasm, it substantially reduced the severity of cortical necrosis (LÁSZLÓ et al., 1966). These observations raise the possibility that hypophysectomy may increase the tolerance to hypoxia, a supposition by no means incompatible with the present results since it is well possible that hypophysectomy is followed by a decrease of cellular sensitivity to hypoxia. If this claim proves true, then we are justified in regarding the absence of ACTH secretion as the primary cause of the reduction in O_2-demand of hypophysectomized animals, since administration of ACTH makes them again susceptible to the injurious effect of hexadimethrine bromide.

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EFFECT OF ACUTE EXPERIMENTAL POLYCYTHAEMIA ON CARDIAC OUTPUT

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The immediate haemodynamic effects of polycythaemia induced by erythrocyte transfusion have been studied in the dog. Cardiac output diminished, while total peripheral resistance increased and blood pressure remained practically unchanged. The arterio-venous O_2-difference invariably increased though venous O_2-saturation showed no significant change. The reduction in O_2-transport was compensated by a corresponding increase in O_2-utilization.

While opinions agree concerning the haemodynamic effects of hypervolaemic anaemia, the opposite is the case with hypervolaemic polycythaemia. An increase in cardiac output has been found by some authors [1, 3, 7], a reduction by others [10, 11]. Certain investigators induced normovolaemic polycythaemia in animals by simultaneous bleeding and transfusion of erythrocytes, but in human pathology polycythaemia is generally associated with hypervolaemia. The present study has been concerned with the haemodynamic effects of polycythaemia induced by transfusion of erythrocytes.

Material and method

Anaesthesia with 0.10 g per kg chloralose was induced in dogs of both sexes ranging in weight between 10 kg and 20 kg, and fasted for at least 18 hours. A cannula was inserted into the trachea and attached to a Krogh-apparatus adopted to estimation of O_2-consumption in dogs. Mean arterial pressure was measured with a mercury manometer in the common carotid. Cardiac output was estimated on the basis of FICK's direct principle. For this purpose arterial blood was taken from the femoral artery, and mixed venous blood from the pulmonary artery through a catheter introduced under radiographic control. Blood clotting was prevented with heparin. Blood samples were collected under paraffin and kept in a refrigerator until the time of study. O_2-saturation of blood was determined by the aid of a Kipp haemoreflector and O_2-content was calculated from the haematocrit and haemoglobin values. Peripheral resistance was computed on the basis of the usual formula: $1332 \times$ arterial mean pressure per cardiac output/60 dyne $\cdot$ cm $\cdot$ sec^{-5} reckoning with zero central venous pressure. The experiments were performed as follows. Cardiac output was estimated together with blood pressure on two or three occasions at 10-minute intervals. In the meantime blood was taken from the donors, the heparinized samples were centrifuged, the plasma was discarded. Of this sample which had now a haematocrit value of 60 to 80 per cent, 300 to 500 ml were introduced into the femoral artery of the test animals at a rate extending the process over 10 to 15 minutes subsequently, at 10 minutes and at one or two further 10-minute intervals, the estimations were repeated.

Results

The overall results have been tabulated. To facilitate comparison, cardiac output was calculated per sq. m body surface, the ratio litre/min/sq. m representing the cardiac index which served as the basis for expressing the total peripheral resistance (TPR) and arterial O_2-transport (product of cardiac index and arterial O_2-concentration). The table presents furthermore the arterio-venous O_2-difference, O_2-saturation of mixed blood, blood pressure, haematocrit and O_2-utilization (A—V O_2-difference arterial O_2-content).

Table 1

	Control	10′ after transfusion	30′ to 40′ after transfusion
Haematocrit %	43.2 ± 5.08	55.0	54,4
		$P < 0.001$	$P > 0.20$
		$P < 0.001$	
Blood pressure, mm Hg	139.0 ± 27.6	135.5	126.6
		$P > 0.30$	$P < 0.02$
		$P < 0.05$	
Cardiac index, 1/min/sq. m	3.25 ± 1.01	2.28	1.73
		$P < 0.01$	$P \sim 0.05$
		$P < 0.001$	
TPR cm · dyne · sec^{-5}	3672 ± 1335	5338	6204
		$P < 0.05$	$P > 0.10$
		$P < 0.001$	
Arterio-venous O_2 difference vol %	4.69 ± 1.20	6.73	8.01
		$P = 0.001$	$P > 0.05$
		$P < 0.001$	
Pulmonary artery O_2-saturation %	69.1 ± 7.38	68.4	60.7
		$P > 0.60$	$P < 0.01$
		$P < 0.01$	
O_2-transport ml/min	591 ± 190	531	400
		$P > 0.20$	$P < 0.05$
		$P < 0.001$	
O_2-utilization	25.7 ± 6.02	28.5	34.7
		$P > 0.20$	$P > 0.05$
		$P < 0.01$	

Under the present experimental conditions, transfusion of erythrocytes reduced cardiac output and increased TPR. Since O_2-consumption was practically unaffected, the increase in A—V O_2-difference was highly significant ($P < < 0.0011$). This was in good agreement with the clinical finding of an increased A—V O_2-difference in polycythaemia even in the absence of circulatory failure. The reduction of O_2-saturation in mixed blood of the pulmonary artery was not of such an extent as to connect the increase in A—V O_2-difference with stagnant hypoxia. The reduction in cardiac output was followed by a minor reduction in O_2-transport. The increase in O_2-utilization (11 and 35 per cent) corresponded to the reduction of O_2-transport (10 and 32 per cent).

Discussion

Goldsmith (1936) could show in polycythaemic patients an increased cardiac output and connected this finding with an increase in blood volume. Stewart et al. (1941) suggested that the augmented A—V O_2-difference was due to the slowing down of circulation caused by the reduced venous O_2-content. In the present experiments this reduction was insignificant in spite of the increased A—V O_2-difference. Regan et al. (1963) have likewise observed a reduced cardiac output associated with reduced coronary flow. The finding of a normal O_2-saturation in coronary sinus blood was regarded by these authors as evidence against the possibility of myocardial hypoxia. It may be of interest to confront these observations with the findings of an increased renal blood flow in polycythaemia vera (Scott et al., 1950; de Wardener, 1951; Malizia, 1956; Doering, 1956), even though these investigations have been carried out under different conditions. Nevertheless, the basic feature, polycythaemia, was common to both.

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RECENSIO

KLOTZ, H. P.: *Les déséquilibres endocriniens*

L'Expansion Scientifique Française, Paris 1966

Monographs on the theoretical or clinical aspects of endocrinology usually treat their subject in the context of the individual glands. The present volume departs from the conventional scheme. Its main sections are devoted to the physiological, biochemical, pathological, and clinical issues of the essential endocrine manifestations, seeking in this manner a synthetic approach to the results of experimental and clinical endocrinology. The least that may be said is that the author has done justice to this task, the introductory section gives a historical outline of endocrinology. The picture of the current state of endocrinological research is particularly illuminating. It gives occasion for stressing the circumstance all too often ignored that outside the hormonal level prevailing in the organism, the responsiveness of the individual effector organs or receptors also determines the character of the endocrine effects. It is, furthermore, justly emphasized that the various neuroendocrine mechanisms operate apart from their regulatory influence on the pituitary gland also through direct nervous pathways to the effector organs.

The following sections deal with the endocrine background of sexual function and of iodine, carbohydrate, fat, and electrolyte metabolism under normal and pathologic conditions. This is followed by the discussion of the hormonal aspects of melanin metabolism, of arterial blood pressure, and calcium balance. Full sections are devoted to the neurohormonal regulations and to the endocrine aspects of ageing. The subsections on the hormonal imbalances in puberty, gestation, and menopause are highly instructive. The last section resumes current views on the arisal of the various types of hormonal imbalance.

The synthetic line of approach has been maintained throughout, and the nonhormonal factors have been duly pointed out wherever necessary. The author would still add to the value of his work by defining the extent of the various endocrine and nonendocrine influences still more closely in an edition to come. The monograph includes 1803 references. A judicious use has been made of long-established facts still of sufficient impact. The author has a brilliant and at the same time lucid style making the subject accessible even to those who are less familiar with the questions of endocrinology. He sets forth his views with a commendable explicitness and presents even controversial issues with a remarkable clarity. A particular asset of the work lies in its excellent illustrations. Almost every aspect of the subject is documented by outstanding single or serial illustrations mostly from the author, a few of them from other sources. By this fact, the monograph has the additional merit of an endocrinological atlas.

KLOTZ's new monograph thus may be summed up as a readable and highly informative work destined for the use of the investigator engaged in theoretical or practical endocrinology as well as of the beginner who seeks basic information on the subject.

I. HOLLÓ

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РЕЗЮМЕ

ИССЛЕДОВАНИЕ ИЗМЕНЕНИЙ РЕФРАКЦИИ

Л. МОЛЬНАР

Автор исследовал у 84 преждевременно рожденных детей, у 81 нормального новорожденного, у 426 грудных детей, у 48 детей младше двух лет, и у всего населения села Уйлета (2084 чел.) на основании преломления света, определенного при циклоплегии, изменения рефракции, наблюдаемые по мере старения, изменения способности роговицы, преломлять свет и частоту случаев миопии, эмметропии и гиперметропии. На основании полученных данных автор устанавливает величину диоптрии средней рефракции при рождении, изменения этой величины по мере старения, срок заканчивания развития оптического аппарата и выясняет процесс эмметропизации. В связи со сравнением данных рефракции 111 косоглазых детей указывается также на этиологию отдельных форм косоглазия.

ШОК И ОТЕК ЛЕГКИХ

И. САМ

Сообщаются клинические и экспериментальные данные о том, что шок может препятствовать развитию отека легких. Казуистика двух случаев демонстрирует, что уже возникший отек легких может полностью регрессировать после появления шока. Встречается, однако, и обратная последовательность возникновения симптомов: у третьего больного острый отек легких развился после преодоления шока. В опытах на 85 крысах было выявлено, что формалиновый шок тормозил развитие экспериментального отека легких, вызываемого впрыскиванием NH_4Cl или вдыханием чистого кислорода под высоким давлением.

САХАРНЫЙ ДИАБЕТ ПРИ НЕДОСТАТОЧНОСТИ ГИПОФИЗА

Э. ГОТ и Дь. МИКЛОШ

У 65-летней мужчины через 15 лет после удаления гипофиза по поводу хромофобной аденомы развилась острый сахарный диабет. Дачей Букарбана удалось поддержать больного в состоянии равновесия. Через $2^1/_2$ года после развития диабета больной умер от цирроза печени, стафилококкового энтероколита и пневмонии. На вскрытии на месте удаленного гипофиза была обнаружена псевдокиста. При гистологическом исследовании в стенке псевдокисты было найдено очень незначительное количество гипофизарной ткани. На основании метопироновой пробы и исследования липоидо-мобилизующего фактора эта ткань еще осуществляла некоторую функцию. В свете этих данных напрашивается вопрос, играет ли гипофиз на самом деле всегда важную роль в патогенезе сахарного диабета.

ДЕЙСТВИЕ ГИПЕРТОНИЧЕСКОГО РАСТВОРА МАННИТА НА КРОВООБРАЩЕНИЕ И НА ФУНКЦИЮ ПОЧЕК В СОСТОЯНИИ ОСТРОЙ ПОТЕРИ КРОВИ

Дь. САБО и Ж. МАДЬЯР

У наркотизированных собак в состоянии гипотонии, вызванной кровопусканием, при величине артериального давления, стабилизированной на 60 мм рт. ст., дача 200 мл 20%-ного раствора маннита оказывает существенное диуретическое действие, инулиновый и ПАГК-клиренсы значительно повышаются, хотя инулин не достигает величин, измер-

яемых в норме. Ретрансфузия взятой крови также повышает диурез, однако, без изменения величин клиренса. При непосредственном измерении под влиянием маннита наблюдается лишь умеренное повышение количества крови, протекающей через почки, причем почечное сопротивление несколько понижается (в среднем на 16%).

При поддержании неизменного уровня артериального давления маннит в значительной мере увеличивает минутный обьем сердца и понижает периферическое сосудистое сопротивление в среднем на 33%.

В экспериментах, в которых авторы в время вливания маннита прекратили кровопускание, артериальное давление повышалось с 60 до 89 ±3,1 мм рт. ст. а минутный объем увеличивался почти до двукратной контрольной величины, измеренной до обескровливания. Общее сосудистое сопротивление понижалось до 35% контрольной величины. Ретрансфузия взятой крови больше не вызывала дальнейшего повышения величины минутного объема.

В этой серии опытов диурез также значительно повышался. Величины клиренса ПАГК и инулина существенно превышали контрольные величины, однако, это было вызвано, по большей части, выделением клиренсовых веществ, накопившихся в почках в состоянии гипотонии.

При непосредственном измерении под влиянием маннита наблюдалось повышение кровообращения в почках до величины, измеряемой при нормальном кровяном давлении. Сосудистое сопротивление в почках значительно понижалось (в среднем на 33%).

Результаты указывают на то, что понижающее сосудистое сопротивление, действие маннита, обуславливается не исключительно лишь уменьшением вязкости крови, оно не представляет собой специфического почечного действия, а проявляется в системном кровообращении еще более выраженно, чем в сосудах почек.

ПРОБЛЕМА РОЖИ II

Свойства стрептококковых штаммов выращенных из патологического материала больных с рожой и другими дерматозами и их роль в патомеханизме заболевания

Й. БЁСЁРМЕНЬИ, Й. СИТА, Э. РАЙКА, Ш. КОРОШИ и М. ГОЗОНЬ

Из патологического материала 115 больных рожой и 214 больных микробной экземой было выращено 66 штаммов β-гемолитических стрептококков. 25 из них относились к группе A, 22 — к группе C и 19 — к группе G. Представители группы A относились к 12 различным типам. У 64 из выращенных штаммов авторы исследовали способность убивать мышей, причем, они в преобладающем большинстве оказались совершенно невирулентными, а меньшая часть их обладала сниженной вирулентностью. Ввиду того, что эти в отношении мышей невирулентные стрептококки вызывали у человека тяжелые болезни, и что из поведения клеточной реакции позднего типа, из гистологической картины и из отсутствия настоящено иммунитета можно было заключить о дефекте иммунитета, можно полагать, что патогенность для человека этих невирулентных штаммов следует приписывать этой слабости иммунитета. Слабое антигенное раздражение и уменьшенная специфическая способность организма вырабатывать антитела, могут, совместно, быть причиной частоты заболеваемости рожой.

ЗНАЧЕНИЕ БАКТЕРИЕМИИ И ТОКСИНЕМИИ В ВОЗНИКНОВЕНИИ ОЛИГО-АНУРИИ

Ф. РЕНИ-ВАМОШ и М. ЧЕЛЛАР

В течение $6^1/_2$ лет авторы наблюдали 87 случаев олиго-анурии бактериемической-токсинемической этиологии. Общая смертность составляла 49,4% (43 случая). Билатеральный некроз коры почек наблюдался в 8 случаях (смертность 100%), шоковая почка была обнаружена в 56 случаях (смертность 32,1%), внезапное обострение хронического заболевания почек было установлено в 23 случаях (смертность 74%). Вызывающими причинами некроза коры почек и шоковой почки были в первую очередь септические аборты, а хронического заболевания почек (прежде всего хронического пиелонефрита) — холецистит, холангит и холангиолит.

ФУНКЦИЯ ПОЧЕК ПОСЛЕ ЛИГИРОВАНИЯ ПОЧЕЧНОЙ АРТЕРИИ

А. ФЕКЕТЕ

У собак после лигирования почечной артерии в случае развития подходящей анатомической системы коллатералей удалось выявить восстановление функции почек. На основании экспериментальных данных весьма вероятно, что для развития коллатерального кровообращения необходимо сохранить в течение известного времени интактную правую почку. По истечении 3 месяцев удаление интактной почки выраженно способствует развитию коллатерального кровообращения и кровоснабжению тканей в почке с лигированной почечной артерией.

Установлено, что уменьшение кровообрашения в почке вызывает и поддерживает в организме животного гипертонию. На основе экспериментальных данных полагается, что по истечении трех месяцев гипертония оказывается вредной для организма, и этому обстоятельству следует приписать, что собаки, которым производили удаление правой почки через 4—6 месяцев после лигирования почечной артерии, погибли от недостаточности почек.

МЕХАНИЗМ ИЗМЕНЕНИЙ ЭКГ ПРИ ТИРЕОТОКСИКОЗЕ

П. ШВЕЙЦЕР, Т. ХИЛЬДЕБРАНД, Х. КЛВАНОВА и Е. МЕРСТЕНОВА

Проводился анализ изменений ЭКГ при тиреотоксикозе и связи между ЭКГ и некоторыми клиническими и гемодинамическими факторами. Полученные результаты показывают, что у больных тиреотоксикозом в вызывании изменений ЭКГ, кроме гиперактивности щитовидной железы, участвуют и другие факторы.

МАТЕМАТИЧЕСКИЙ АНАЛИЗ ТЕОРИИ СТАРЛИНГА О ВОЗНИКНОВЕНИИ ОТЕКОВ С УЧЕТОМ ГЕМО-ЛИМФОДИНАМИЧЕСКИХ СООБРАЖЕНИИ

М. ФЁЛЬДИ и Л. ЛЕХОТАИ

На основании теории Старлинга в возникновении водянки рост капиллярного давления и уменьшение коллоид-осмотического давления имеют нумерически одинаковое значение.

С учетом лимфодинамических условий авторы настоящей статьи выдвигают теорию, что в случае генерализованной флебогипертонии повышение капиллярного давления представляет собой в два раза большую гидропигенную силу, чем уменьшение коллоидно-осмотического давления.

Модифицированную авторами теорию, как и классическую теорию Старлинга можно выразить в уравнениях.

На основе данных 110 больных *Фаркаша* и *Кораньи*, без водянки и с водянкой различной степени и различного генеза, удалось установить новые математические зависимости и то, что, соответственно теории авторов, при генерализованной флебогипертонии гидропигенное значение капиллярного давления превышает значение коллоидно-осмотического давления на самом деле в два раза.

ДЕЙСТВИЕ ГЕНЕРАЛИЗОВАННОЙ ФЛЕБОГИПЕРТОНИИ НА ЛИМФООБРАЩЕНИЕ И НА ВОЗНИКНОВЕНИЕ ОТЕКА В ОПЫТАХ НА МОДЕЛИ

М. ФЁЛЬДИ, А. ЛАКОШ, Л. ЛЕХОТАИ и Ш. ШОНКОДИ

На простой гемо-лимфодинамической модели можно установить, что в гидропигенном действии флебогипертонии, генерализовавшейся вследствие действия физических сил, значительную роль играет то обстоятельство, что повышение давления, распространяясь обратно на лимфатическую систему, препятствует впайению лимфы. На модели наглядно видны также принципиальная разница между «закрытым» и «канюлированным» лимфотоками и связь между возникновением отека и расстройством лимфообращения.

ПОГЛОЩЕНИЕ РАДИОЦИНКА ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗОЙ ПРИ ЕЕ ОСТРОМ ПОВРЕЖДЕНИИ

Г. МАКАРА

Исследовалось поглощение Zn^{65}-глицината и Zn^{65}-глюконата щитовидной железой, печенью и тощей кишкой белых крыс. Через 1—7 часов после впрыскивания наблюдалось в поджелудочной железе подопытных животных значительное поглощение радиоцинка. После лапаротомии поджелудочная железа крыс поглощала меньше, а их печень поглощала больше радиоцинка, чем соответствующие органы контрольных животных. При дезоксихолатовом панкреатите поджелудочная железа и тощая кишка животных поглощает еще меньше изотопов, чем после лапаротомии. Уменьшение поглощения радиоцинка объясняется, по всей вероятности, ухудшением кровоснабжения воспалительной поджелудочной железы.

ДЕЙСТВИЕ ГИПОФИЗЭКТОМИИ И ДАЧИ АКТГ НА ИЗМЕНЕНИЯ, ВЫЗВАННЫЕ БРОМИДОМ ГЕКСАДИМЕТРИНА

К. КОВАЧ, И. СИЙ, Ю. КОЧИШ и Ф. ЛАСЛО

После дачи бромида гексадиметрина температура прямой кишки гипофизэктомированных крыс резко понижается и в пределах нескольких часов большинство животных погибает. Некроз внутренних слоев коры надпочечников, а также восходящей ветви петлей Генле менее выраженный, чем у контрольных животных. Инфаркта печени и кальциноза почек не развивается. Некроз клубочковой зоны же закономерно появляется. Если через день после гипофизэктомии животным давали АКТГ, то эта предварительная дача приводила к умерению понижения температуры прямой кишки, наблюдаемого после введения бромида гексадиметрина, к удлинению времени выживания животных, при одновременном увеличении некрозов, возникающих во внутренней корковой части надпочечников, в почках и в печени. Спустя месяц после гипофизэктомии крысы реагировали в меньшей мере действие АКТГ в случае дачи бромида гексадиметрина. У этих животных введение АКТГ не предотвращало понижения температуры прямой кишки, не вызывало существенного изменения времени выживаемости крыс. С другой стороны АКТГ обусловливает усиление некрозов в органах. Эти результаты указывают на то, что в случае дачи бромида гексадиметрина гипофизэктомия имеет двоякий эффект; с одной стороны, она понижает сопротивляемость крыс, а с другой — предоставляет определенную защиту против развития повреждения тканей.

ДЕЙСТВИЕ ОСТРОЙ ЭКСПЕРИМЕНТАЛЬНОЙ ПОЛИГЛОБУЛИИ НА МИНУТНЫЙ ОБЪЕМ

З. НАДЬ, И. ЯКАБ, А. МЕСАРОШ, Ш. ФЮЛЁП, А. КАРАИ и И. ЛУЛИТЬ

В острых опытах на собаках авторы исследовали действие трансфузионной полиглобулии на кровообращение и установили, что минутный объем уменьшился, а сопротивление кровообращению повысился при незначительном изменении кровяного давления. Рост артерио-венозной разности кислорода был нормальным, без значительного уменьшения кислородной насыщенности венозной крови. Уменьшение кислородного транспорта компенсируется соответствующим ростом использования кислорода.

XIth CONGRESS OF THE MEDICAL WOMEN'S INTERNATIONAL ASSOCIATION
XIème CONGRÈS DE L'ASSOCIATION INTERNATIONALE DES FEMMES MÉDECINS
KONGRESS DER INTERNATIONALEN VEREINIGUNG DER ÄRZTINNEN

Information:

The XIth CONGRESS OF THE MEDICAL WOMEN'S INTERNATIONAL ASSOCIATION will take place in Vienna/Austria from JUNE 23 to JUNE 29, 1968.

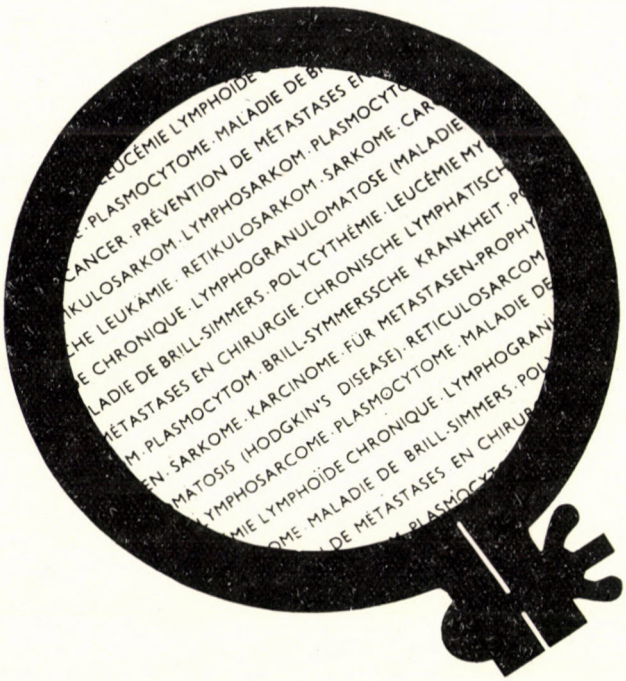
The general topic will be: "The Hungry Millions".
The Congress is open to all women physicians of the world.
A First Preliminary Programme will be available this summer.
For information please apply to: Secretariat XIth Congress of the Medical Women's International Association, c/o Wiener Medizinische Akademie, Stadiongasse 6—8, A 1010 VIENNA/Austria

Le XIème CONGRÈS DE L'ASSOCIATION INTERNATIONALE DES FEMMES MÉDECINS aura lieu à Vienne/Autriche du 23 au 29 JUIN 1968.

Le thème général du Congrès sera «Les Millions d'Affamés».
Le Congrès sera ouvert à toutes les femmes médecins du monde entier.
Un 1er Programme Préliminaire sera imprimé au cours de l'été 1967.
Pour toute information veuillez vous adresser au: Secrétariat XIème Congrès de l'Association Internationale des Femmes Médecins, c/o Wiener Medizinische Akademie, Stadiongasse 6—8, A 1010 VIENNE/Autriche

Der 11. KONGRESS DER INTERNATIONALEN VEREINIGUNG DER ÄRZTINNEN findet vom 23—29. JUNI 1968 in Wien statt.

Das Leitthema des Kongresses lautet: »Die hungernden Millionen«.
Alle Ärztinnen der Welt können an diesem Kongress teilnehmen.
Ein 1. Vorprogramm wird im Laufe des Sommers 1967 erscheinen.
Zuschriften sind zu richten an das Sekretariat 11. Kongress der Internationalen Vereinigung der Ärztinnen, c/o Wiener Medizinische Akademie, Stadiongasse 6—8, A 1010 WIEN/Österreich



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