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X-RAY CHANGES IN POLYCYTHAEMIA VERA

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(Received May 27, 1972)

Routine chest X-rays have been evaluated in 49 cases of polycythaemia vera Abnormal radiological features resulting from the modified haemodynamic conditions, in other words, from the primary disease itself, occurred in 35 cases. The frequency of the changes showed a direct relationship with the stage of activity of the disease. With the exception of pulmonary fibrosis, the changes proved reversible. The X-ray signs found during relapses subsided or disappeared during remissions.

Polycythaemia vera represents a particular type of polyglobulism in which no primary factor or primary process can be demonstrated: the disease consists in the polyglobulism [9]. The disease is marked by a proliferation of all the three haemopoietic, i.e. erythro,-leuko-and thrombopoietic, elements of the bone marrow. This determines its most prominent haematological features: an increase in erythrocyte count, haematocrit value, blood volume and generally also in the leukocyte and platelet counts. Blood viscosity is invariably increased. These haematological abnormalities account for the classical clinical syndrome including thromboembolic complications and haemodynamic changes characterized by a slowing of circulation, congestion and vasodilatation. The associated cardiorespiratory symptoms and the corresponding findings of blood-gas analysis [8, 10] are due to these haemodynamic changes.

Though it is common knowledge that haemodynamic disorders of the lesser circulation cause X-ray changes in the pulmonary parenchyma, the heart and the large vessels, there are few reports on X-ray observations of a large material of polycythaemia vera followed up for longer periods [4, 5, 6, 11].

There are, basically, two reasons for which X-ray studies in polycythaemia vera might be of importance. First, the typical X-ray changes may help to differentiate polycythaemia vera from secondary polycythaemias. Second, if the syndrome is associated with X-ray signs of corresponding severity, then the possible changes in these signs may be regarded as an index of the haematological response, of the efficiency of treatment.

On the grounds of these considerations it seemed interesting to examine the chest X-ray changes in a large series of polycythaemia vera patients.

The aim of these studies was to clarify the incidence of X-ray changes in polycythaemia vera as well as the nature and association of these signs. The study was also expected to answer the question how far the X-ray signs are correlated with the haematological findings and the duration of the disease.

Material and methods

In the past two years 57 patients with polycythaemia vera confirmed on haematological and clinical grounds were submitted to chest X-ray studies parallel with examinations of the general state, blood counts, bone marrow smear, haematocrit and haemoglobin values.

In the history of 8 patients there were various pulmonary diseases (tuberculosis, exudative pleuritis, chronic bronchitis, bronchiectasis, etc.) which had left permanent changes interfering with proper interpretation. These cases were excluded from the analysis.

The remaining 49 patients were divided into two groups according to whether — on the ground of clinical and haematological features — they were in relapse or in remission. At the time of the first study 33 patients were in relapse, their mean counts being RBC 6,461,000; WBC 10,700; 430,000 platelets. Sixteen patients were in remission, the respective values being 4,393,000; 6,700 and 192,000.

The investigations were repeated 6 to 12 months later in 27 cases. Of these patients, 7 were in relapse on the first, and in remission on the second investigation, for 3 patients the inverse was true. Thirteen patients were in relapse, four in remission on both occasions.

At the time of the first investigation the polycythaemia had been present for 0 to 5 years in 24, for 6 to 16 years in 21, and longer than 10 years in 4, cases.

Twenty-six patients were females, 23 were males. Their age ranged from 19 to 76 years, the majority of the patients were between 40 and 60 years of age.

Eighteen patients had arterial hypertension responding to the conventional antihypertensive agents.

The radiological examination comprized chest X-rays in two views, fluoroscopy combined with a barium meal for visualization of the oesophagus. This was completed by kymography in individual cases. Attention was given in every case to the pars intermedia of the pulmonary artery situated in the right hilum, its significance has been pointed out by ASSMANN as a reliable index of pulmonary arterial pressure [1].

Results

The X-ray features, their association and their relationship with the prevailing stage of the process are shown together with the clinical diagnosis in Table I.

According to the literature [4, 6, 11] confirmed by our own observations the most typical X-ray features associated with polycythaemia vera

Table I

Phase of disease	X-ray signs						Dilatation of pars intermedia
	Number of cases	typical	suggestive	nontypical	total	none	
Relapse	33	24	2	2	28	5	16
Remission	16	2	2	3	7	9	5
Total	49	26	4	5	35	14	21

include enlargement of the heart to the left, distinct concavity of the sinus, prominence of the aortic knob, moderate symmetrical hilar enlargement and hypervascularization of the lungs. These signs have been summarized under the heading typical X-ray features in Column 3. Column 4 represents signs suggestive of the disease, thus hypervascularization in the presence of a normal heart and aorta, and Column 5 shows those individual changes of the heart and aorta which form part of the typical syndrome. Column 6 represents the cases in which the pars intermedia of the pulmonary artery was more than 14 mm in diameter.

As Table I shows, investigations revealed X-ray changes related to the primary disease in 35 patients, more than two thirds of the cases. X-ray changes were demonstrable in 80% of the cases in relapse and in 50% of those in remission. Twenty-six X-rays showed all features of the typical syndrome (Fig. 1).

V. Gy., a 46-year-old female patient, had been under treatment for 7 years. She is now in a relapse.

Anteroposterior X-rays revealed a moderate enlargement of the heart to the left, with a distinct concavity of the sinus and a slight prominence of the aortic knob. The central branches of the pulmonary artery are congested, the hila slightly enlarged. There are numerous perihilar point-like vascular

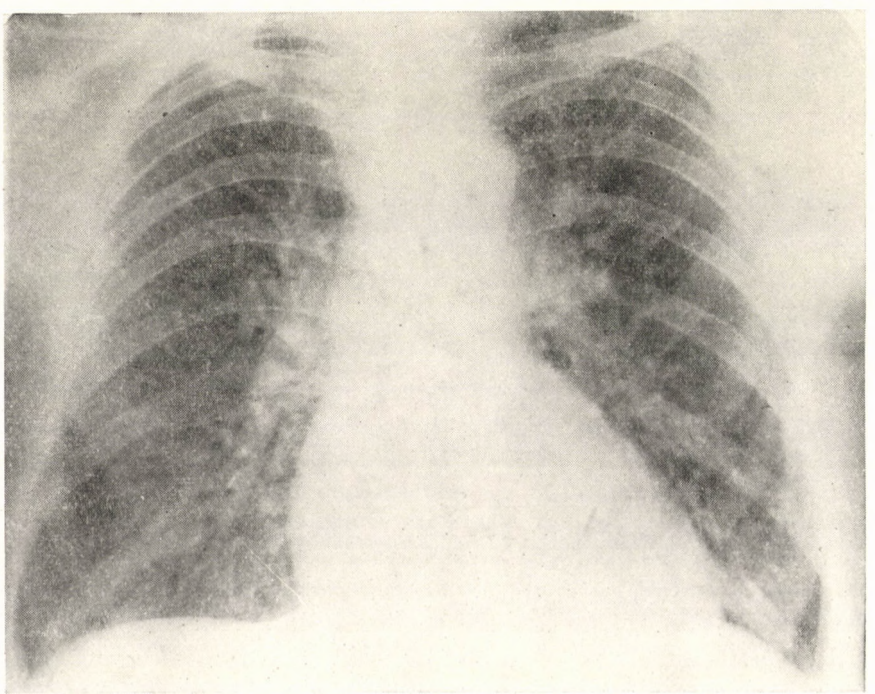

Fig. 1 (Case 1). Chest X-rays. Typical polycythaemia vera syndrome in relapse

densities. The hilar vessels are sharply outlined, their branches can be followed up to the periphery.

In 4 cases hypervascularization of the lung was not associated with any abnormality of heart and aorta.

In 21 cases the pars intermedia measured more than 14 mm in diameter (the normal range given by ASSMANN [1] is from 9 to 14 mm).

Fourteen of the 49 patients failed to reveal any morphological change related to polycythaemia vera. These cases included 5 patients in relapse and 9 in remission. This also illustrates the fact that polycythaemia vera, even in the period of recurrence, need not be associated with abnormal chest X-rays [6, 11].

Table II sums up the relationships between the presence of the typical syndrome, including dilatation of the pars intermedia and pulmonary fibrosis, on the one hand and the duration of the disease on the other.

Table II

History	Number of cases	Typical syndrome	Dilatation of pars intermedia	Pulmonary fibrosis
0 to 5 years	24	11	9	3
6 to 10 years	21	11	8	6
Longer than 10 years	4	4	4	4
Total	49	26	21	13

Though, as shown in Table II, the frequency of the typical syndrome is related to the haematological status, in other words, to the prevailing stage of activity, it is none the less a fact that in the four cases where the disease had been present for more than ten years, the typical signs were marked.

This was true also for the relationship between pulmonary arterial dilatation and duration of the disease.

The incidence of pulmonary fibrosis involving the basal region of the lungs in the form of fine reticular densities was also related to the duration of the disease.

In opposition to RICHTER [11] we found no Kerley lines in this series.

As mentioned earlier, the studies were repeated 6 to 12 months later in 27 cases. The results allowed to assess the changes in the X-ray findings in the light of those of the haematological status.

In 5 patients who were in relapse when examined for the first and in remission when seen for the second time, a considerable, in three of them even complete, regression of the X-ray abnormalities was demonstrable.

In 2 of the three patients having been in remission with normal X-rays when seen for the first, and in relapse when seen for the second time, the second X-ray study revealed a typical syndrome and in the third patient, pulmonary hypervascularization. Thirteen patients were in relapse on both occasions. In 12 of these the X-rays remained practically unchanged, whereas in one with originally normal findings, a distinct hypervascularization of the lungs was demonstrable on the second occasion. In the 4 patients who had been in remission on both occasions there was practically no difference in the results of the two studies.

The individual X-ray abnormalities and their changes may be summed up as follows.

Cardiac enlargement, increased concavity of the sinus, elongation of the aorta, prominence of the aortic knob were of minor degree in most cases. These signs are rarely encountered during remission, and even in relapse they usually remain absent until signs of hypervascularization become manifest. A heart of aortic configuration with marked left ventricular enlargement was only seen in cases associated with arterial hypertension. X-ray abnormalities of this type would remain unchanged in clinical remission. Enlargement of the right ventricle was confined to cases of marked pulmonary fibrosis. Hypervascularization of the lungs is a typical and common feature which may, however, be obscured by the presence of pulmonary fibrosis on films taken with conventional techniques. The most distinctive sign of hypervascularization is the sharp demarcation of the distended vessels which can easily be followed up to the periphery. There are also multiple point-like orthoroentgenograde densities. The regularity of ramifications tapering off toward the periphery are suggestive of vessels rather than of fibrosis. Vascularization has a wide range of individual varieties depending on constitutional factors and its X-ray appearance may be different in the successive phases of respiration even in the same individual. The most characteristic change in the X-ray picture parallel with a favourable haematological response was a clearing up or the disappearance of hypervascularization. An associated lesser circulatory failure blurs the outlines of the hilar and peripheral vessels and makes proper interpretation difficult. However, in the present series hilar enlargement and dilatation of the pars intermedia was of minor degree all throughout and the diameter of this section of the pulmonary artery was never in excess of 18 mm. Active pulsation of the sharply outlined distended central vessels was confirmed by kymography. A distinct reduction in the caliber of these vessels was demonstrable in several cases during remission and it was only in the presence of age-related sclerosis that the abnormalities in question were independent of the activity of the primary disease.

The X-rays presented in Figs 2/a and 2/b permit to follow up the changes in caliber of the pars intermedia associated with the clinical response.

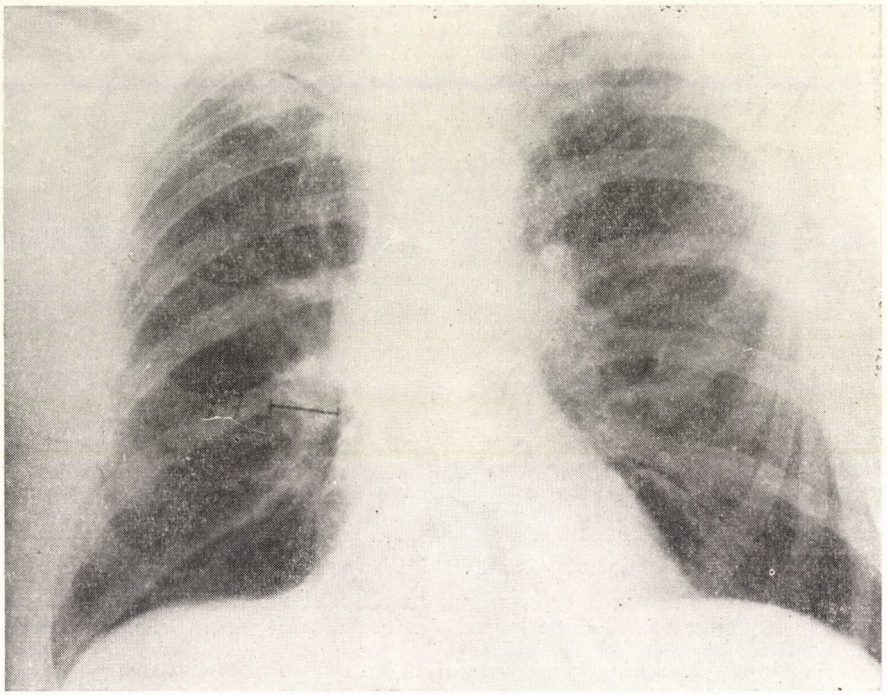

Fig. 2a (Case 2). Chest X-rays. Pars intermedia 17 mm in diameter

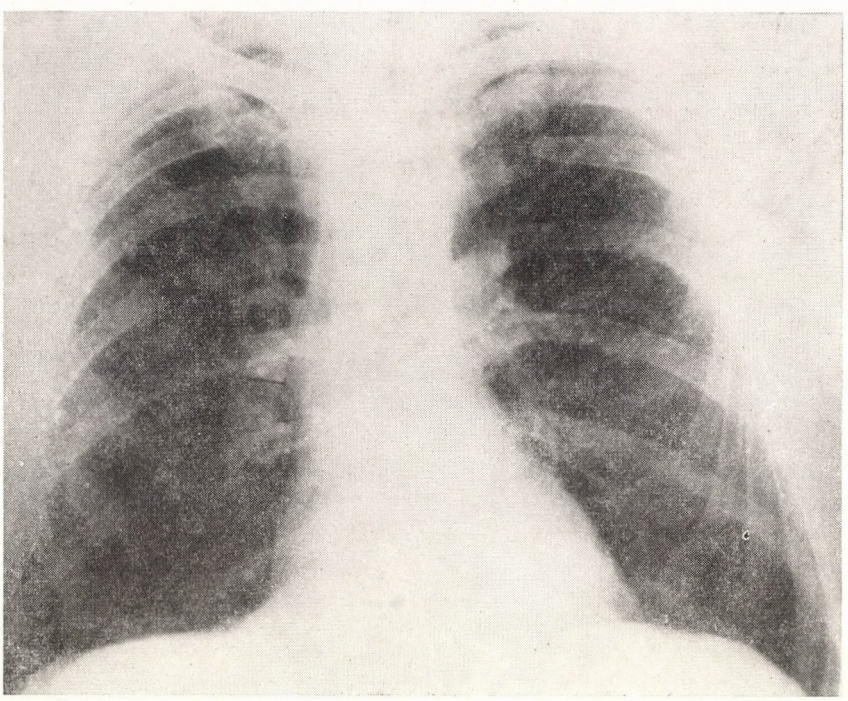

Fig. 2b (Case 2). Chest X-rays in remission. The pars media has diminished to 13 mm in diameter

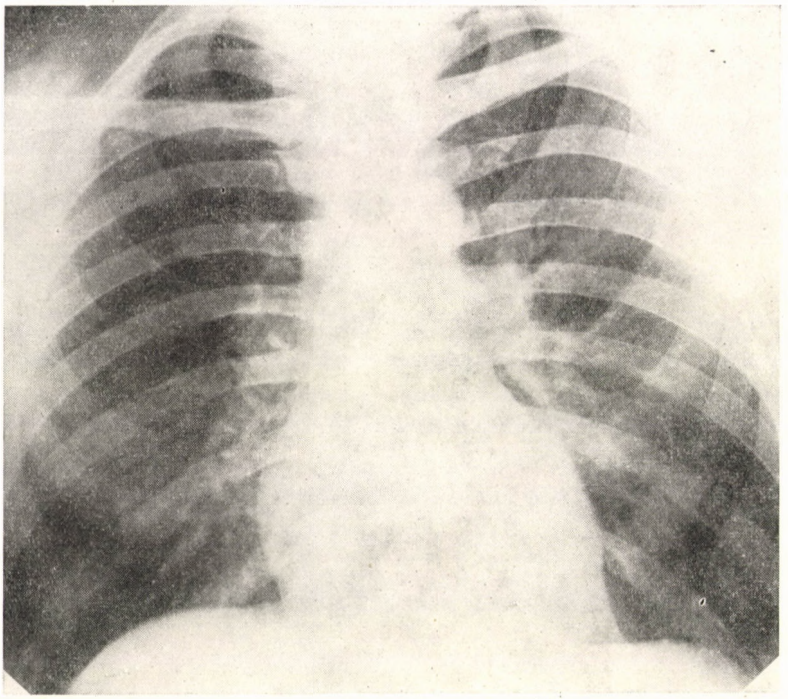

Fig. 3 (Case 3). Chest X-rays. Typical polycythaemia vera syndrome with pulmonary fibrosis

G. E. was a 47-year-old male patient in whom polycythaemia vera had been diagnosed three years earlier. X-rays made in a period of relapse (a/a) showed all features of the typical syndrome. The pars intermedia of the pulmonary artery measured 17 mm in diameter according to the criteria set by ASSMANN. X-rays made 9 months later in a period of remission (2/b) showed that hypervascularization had definitely subsided, the pars intermedia diminished to 13 mm in diameter and hilar enlargement was less marked. Pulmonary fibrosis was generally associated with processes of long standing or occurred in patients whose early treatment had been neglected or in cases of utmost severity accompanied by repeated relapses or heart failure. Fibrosis was always of permanent nature. If of major extent, it resulted, similarly to other processes associated with a loss of pulmonary parenchyma, in precapillary pulmonary hypertension and cor pulmonale which in our experience may be a source of misinterpretation by obscuring the X-ray features of polycythaemia vera.

The film in Fig. 3 shows a case of polycythaemia vera associated with pulmonary fibrosis.

T. D., a 62-year-old male patient, had had polycythaemia vera for 13 years. At the time of study he was in relapse.

X-rays showed the heart moderately enlarged to the left, a markedly concave sinus, a slightly prominent aortic knob. The basal areas of the lungs showed fine fibrous bands which, together with the increased vascular markings decreased the radiolucency of the basal pulmonary areas. The film clearly shows the radiomorphological differences between the fibrous bundles and the vascular shadows.

Discussion

BREDNOW [2] was the first to describe the typical X-ray abnormalities in polycythaemia vera having noted in 4 cases hilar enlargement with sharply outlined pulmonary arterial branches readily traceable to the periphery. He also furnished kymographic evidence that hilar enlargement was produced by the dilated central branches of the pulmonary artery. Since this paper, the textbooks [12, 13, 14], in discussing the differential diagnostic aspects of pulmonary congestion, have been referring to an increased pulmonary density in polycythaemia vera. However, we find but sparse data in the literature on the frequency, types and differential diagnostic value of these changes.

HODES and GRIFFITH [5] found a normal heart in their cases of polycythaemia vera. HODGSON et al. [6] reported on pulmonary congestion and fibrosis in 25 out of 35 cases. A moderate enlargement of the heart to the left was also noted by these authors in a number of their cases.

RICHTER [11] found uniform pulmonary hypervascularization in 38, enlargement of the pars intermedia in 29, and prominence of the aortic knob in 43, out of 50 cases. The heart was moderately enlarged to the left in every patient. DELAMORE et al. [4] observed focal polycythaemia vera patients in the course of a long-term follow-up.

The present results assessed in the light of data in the literature and of certain haemodynamic considerations allowed to connect the X-ray features directly with polycythaemia vera in 35 of the 47 cases. In 26 cases pulmonary hypervascularization, slight concavity of the sinus, elongation of the aorta and prominence of the aortic knob were found. These signs may be considered typical. Enlargement of the pars intermedia was demonstrable in 21 cases.

The X-ray abnormalities under discussion remain reversible for a long time, corresponding to the activity of the process, subsiding parallel with the responses of the haematological status, particularly in young individuals where degenerative factors have not yet exhausted the adaptability of the organs and tissues. Reversibility of these features is reflected by the substantial difference in the incidence of these abnormalities in relapse and in remission, as well as by a regression or complete disappearance of the abnormalities during periods of remission. Despite these facts we are far from asserting that

there is any linear correlation between clinical severity and the presence of, or the changes in, the X-ray signs. In fact, in a number of patients no X-ray symptom was present during remission, whereas in two cases a typical syndrome was demonstrated during remission. It must be noted that in the former cases the disease had been present for one or two years, whereas in the other two for 8 and 10 years.

Pulmonary fibrosis, demonstrated in 13 of the present cases, is a permanent change of slow progression primarily associated with long-standing cases of polycythaemia vera. The haemodynamic factors involved in its pathomechanism including an increase in blood-volume, slowing of circulation, pulmonary plethora and a failure of the lesser circulation, were regarded by NAGY [7] as a fairly common feature of polycythaemia vera.

As regards the differential diagnostic value of X-rays, changes similar to the observed ones may result from numerous other primary processes such as congenital valvular defects associated with a left-to-right shunt (atrial or ventricular septal defect, patent ductus arterious), diverse forms of circulatory hyperkinesia, as may be found in hyperthyroidism and anaemia, and even in the absence of any demonstrable primary cause, furthermore in association with pulmonary congestion and with an active hyperaemia of the bronchial vessels, e.g., in pulmonary atresia or Fallot's tetralogy.

Among all these, it is a left-to-right shunt of minor degree and an essential circulatory hyperkinesis which may pose serious differential diagnostic problems. In the first case the heart's configuration (right ventricular hypertrophy and dilatation, prominent pulmonary arc), and in the second, absence of signs of any marked severity and the normal configuration of the heart, will decide the issue. Still there are cases in which the X-rays are of little diagnostic aid and the diagnosis has to rely on clinical and haematological evidence.

Despite these facts, a typical association of the characteristic features will confirm the clinical diagnosis of polycythaemia vera in many cases on the sole evidence of routine X-rays. Therefore, in the case of a suspected polycythaemia vera, among the various clinical tests, X-rays should have their place with the understanding, however, that a negative X-ray finding by itself does not invalidate the diagnosis of polycythaemia vera.

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IRRADIATION OF THE THYMUS IN RHEUMATOID ARTHRITIS

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In view of the involvement of the thymus in autoimmune processes, the therapeutic effect of thymus irradiation was observed in 51 patients with rheumatoid arthritis. On the grounds of the favourable results, irradiation of the thymus is regarded as a useful adjuvant procedure in the management of rheumatoid arthritis. In 66% of the cases the effect of treatment was not limited to a short-term benefit but was still demonstrable, on the evidence of objective findings, on reexamination 6 months and 2 years later. Patients of advanced age were found more responsive than juveniles where the response was usually poor.

Recent progress in immunology has given a great impetus to thymus research. The central part played by this organ in the maturation and activity of the immune system has been established by experimental evidence. The organ preserves its role even after its involution in adult age. It is usually enlarged in autoimmune diseases, presumably because of its involvement in these processes [10, 16, 17, 18]. Mackay and De Gaill [15] have performed thymectomy with benefit in rheumatoid arthritis (RA), the intervention having been prompted by their findings of thymic changes in that disease. It is, on the other hand, well-known that surgical removal of the thymus because of hyperplasia or adenoma in myasthenia gravis may be followed by the production of autoimmune disease. A source of contradictions lies in the versatile character of the part which the thymus is assumed to play in autoimmune regulations. Though there is disagreement on the interrelations of the cells being formed in the thymus or passing through it, the fact that the cells acquire their immuno-competence in the thymus stands beyond doubt [4, 5]. This strongly points to a mediatory role of the thymus in autoimmune diseases, as suggested by the presence of germinal centres not only at an experimental level in NZB mice with autoimmune diseases but also in human pathology. Microscopic changes of this kind must always be regarded as evidence of some autoimmune disease [4, 5, 12, 15].

The fact that the lymphoid cells are closely involved in autoimmune processes, for instance in RA, and that their involvement stems in all probability from the part played by the thymus in these conditions, gives a sound basis to the therapeutic irradiation of the organ in these diseases. This

was first advocated for RA by FARKAS [7], in the first place in the presence of enlarged lymph nodes. The response to irradiation of the thymic region in occasional cases of grave, steroid-resistant RA with lymphoid hyperplasia was likewise favourable [8, 21]. This has prompted further observations on a larger scale.

In our opinion, irradiation of the thymic region with lower doses than those used for tumours, is preferable to thymectomy [19], since complete removal of the organ may give rise to a counter-regulatory immune mechanism of adverse effect on the further course of the process. This is at least our inference drawn from two cases of RA where X-ray therapy for breast cancer amounting in reality to radiothymectomy, was followed by a spectacular deterioration of RA. In opposition to this, the radiation doses of 850 to 1200 R advocated by us, while producing no irreversible damage to the tissues, have the advantage over surgical therapy to be operative even in the presence of aberrant mediastinal thymic tissue.

Fifty-one patients with RA had irradiation of the thymus between 1968 and 1970. All had been assigned to the group of "certain" diagnosis by combining more than six characteristic features of the disease. The process was of major severity all throughout, all patients being steroid-dependent and in Steinbrocker's stages II or III. There were 7 males and 44 females between 9 and 79 years of age, average age being 50 years. Three patients were between 9 and 20, 7 between 21 and 40, 23 between 41 and 60, 18 over 60 years of age. Duration of disease ranged from six months to 35 years (average: 9.8 years). 39 patients were seropositive (Latex-positive) for rheumatoid factor, 12 were seronegative.

The characteristics of irradiation applied to the mediastinum were, 160 kV, 10 mA, focal-skin distance 40 cm, half value layer of 0.5 mm Cu, field size $4 \times 10 \times 15$ cm. The total dose was 850 R in the majority of the cases, two patients having been given 750 R and two others 1200 R. Average duration of treatment was 10 days. The doses were increased from 50 to 100, finally to 150 R.

The patients remained on their former drug treatment during radiotherapy. After its completion 24 patients were given azathioprine in doses of 2.5 mg per kg body weight daily over several months. Success of treatment was assessed on the basis of the following criteria.

a) Laboratory parameters. Among these, a fail in ESR and the improvement of anemia proved the best indicators of a favourable response. Serum tests for rheumatoid factor, paper electrophoresis, as well as skin sensitivity tests for tuberculin and for other bacterial and mycetic antigens were performed in every case. In the majority of cases immune-electrophoresis was also carried out. The lymphocyte transformation test was confined to individual cases.

b) The decisive *clinical parameters* include the number of affected joints, improvement in locomotion, in the power of manual pressure, subjective relief, possibility of reducing the corticosteroid doses. In order to check the long-term results, the patients were reexamined six months and two years later.

Results

The results of irradiation of the thymic region assessed on the basis of the foregoing criteria are presented in Tables I and II. Sixty six per cent of

Table I

Results of irradiation of the thymus in rheumatoid arthritis

Early results

Number of patients	Improvement			
	Marked	Fair	None	Deterioration
51	20	16	11	4

Total number of responsive cases: 36
Total number of unresponsive cases: 15

Long-term results

Number of patients	Improvement			
	Marked	Fair	None	Deterioration
40	16	9	9	8

Total number of responsive cases: 25
Total number of unresponsive cases: 15
Note: In 24 out of 40 patients presented under the heading "Long-term results" cytostatic treatment had been applied in addition to radiotherapy. These cases are included in Table II.

the patients have thus been found to respond to thymic irradiation. In view of the spontaneous changes, in the activity of RA, as reflected by the clinical states and by other parameters including ESR, adequate long-term follow-up was considered essential, therefore the patients were reexamined six months and two years after irradiation. The benefits of treatment were still demonstrable on these occasions in 66% of the cases.

Table II

Long-term results of irradiation alone and in combination with cytostatic therapy

Improvement

Irradiation of the thymus Number of patients	Improvement			
	Marked	Fair	None	Deterioration
16	5	4	3	4

Total number of responsive cases: 9
Total number of unresponsive cases: 8

Irradiation + cytostatic therapy Number of patients	Improvement			
	Marked	Fair	None	Deterioration
24	11	5	4	4

Total number of responsive cases: 16
Total number of unresponsive cases: 8

Discussion

Irradiation of the thymus was first undertaken by HALSTED [11] for Graves' disease in 1915. At present, irradiation of the thymus, either in itself or in combination with thymectomy, prior to the operation or consequent upon it, belongs to the current therapy of myasthenia gravis [9, 13]. In the available literature we find reference to irradiation of the thymus in RA. Considering, however, that the general effects of radiotherapy are less adverse than those of the other immunosuppressive procedures, it seemed promising to study the value of irradiation in RA.

The results raise the question whether the thymus of the patients under study was functioning at all and whether the benefits of treatment should not be ascribed to the general effects of radiation. In order to be able to rule out this possibility, we applied the same radiation doses to the sacro-iliac region in three and to the spleen in further three cases. Not only did we fail to note any benefit but there was even a deterioration of arthropathy in the three cases where the spleen had been irradiated. Though the current laboratory investigations had been performed in the majority of the cases, none of the parameters thus obtained were apt to furnish any reliable indication of possible changes in the immune status. Nor did we note any change in those immunological parameters which had been examined in individual cases. For

instance, the lymphocyte transformation test which had been performed in a few cases yielded no information which might have been regarded as conclusive. It is true that in response to irradiation there was a more significant fall in the number of phytohaemagglutinin-sensitive lymphocytes than in the absolute lymphocyte count, but this was so also in the case of irradiation of the spleen which was followed by a clinical relapse. On the other hand, no similar changes were noted after irradiation of the sacro-iliac region.

The radiation doses employed in the present study were lower than those applied for thymic tumours [21]. This had its good reasons. We have referred to our observations relative to the consequences of tumour doses earlier in this report. This fact in itself would strongly suggest that the thymus must be involved in autoimmune diseases in different ways [6], which would account for the inconsistency of published evidence. For instance, HOWIE and HELYER [12] noted an intensification or multiplication of autoimmune manifestations, in particular a rise in the antinuclear factor titres after thymectomy in NZB mice with autoimmune disease. Manifestation of thyroiditis and of SLE as well as positivity of RF and of ANF have been described in man after thymectomy for myasthenia gravis [1]. In opposition to this, other authors, including OSSERMANN and WEINER [20] failed to note improvement of these serological changes, likewise after thymectomy performed for myasthenia gravis. These observations thus appear to be entirely opposed to each other. There is no way of telling which of them is true unless we should be able to compare age-matched thymectomized subjects of identical immunological status. So long as we are unable to satisfy these conditions, we have no possibility of rejecting any of the seemingly contradictory observations. Each may be true in its own right. It must be borne in mind that the characters of immune diseases and immune states are extremely variable, depending on the time of onset, on the intensity as well as on the site of immune manifestations, moreover, on diverse influences on the part of the organism. The same intervention may have different consequences in a different immune status or under different immune conditions. For instance, it may activate some of the humoral or cellular factors of the immune process while suppressing some others. The therapeutic response to an identical radiation dose delivered to the thymus thus seems to vary with the prevailing immunological constellation. Under these aspects, irradiation of the thymus in RA may not be equivalent to a suppression brought to bear on the immunocompetent cells of the organ but would rather seem to represent an intervention which, though being doubtlessly suppressive, may none the less well be apt to elicit an immune regulation of clinical benefit, if the thymus happens to be in a phase suitable for this response.

As regards the present patient material, a long-term benefit has been obtained in more than 50% of the cases. Our observations seem to suggest

that responsiveness of children differs widely from that of subjects of advanced age. While in children the response, if any, was generally poor and transitory, in older subjects with RA of major severity it was striking and of fairly long duration, although irradiation had been the only treatment applied. This has prompted us to apply increased or repeated radiation doses in children, in accordance with the relatively larger size of their thymus. These attempts proved likewise unsuccessful.

The results of KOLLER [14] are apt to throw light on the possible causes of failure in children. It has been demonstrated by this author that regeneration of the thymus consequent upon its radiation-induced atrophy comprizes two phases, the second phase being associated with a repopulation by cells from the bone-marrow. Unresponsiveness to radiotherapy in childhood may thus be accounted for by the larger size and the greater capacity for regeneration of their thymus [3]. It is felt, however, that irradiation of the thymus combined with cytostatic therapy might be more successful in view of its suppressive effect on regeneration. Further observations will have to clarify this point. At any rate, increasing the radiation doses is limited by the well-known hazards [2].

The present observations show that irradiation of the thymus is a useful procedure of durable benefit, as confirmed by follow-up studies after six months and two years. Combination of the procedure with cytostatic therapy might help to attenuate recurrences of RA and to prolong the periods of remission. In the present material azathioprine has been used for this purpose.

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EVALUATION OF THE LABORATORY PROCEDURES FOR THE DIAGNOSTICS OF DISSEMINATED INTRAVASCULAR COAGULATION (DIC) ON THE BASIS OF A MODEL EXPERIMENT

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A mild form of disseminated intravascular coagulation (DIC) was induced in rabbits by a single injection of endotoxin in order to examine the informative value of the laboratory procedures used in the diagnostics of this clinical condition. The variations of the coagulation factors of the plasma were studied, the ethanol-gelation test was also evaluated. The fibrinogen degradation product (FDP) was measured in plasma and serum by immunoelectrophoresis.

Of all parameters studied, the platelet count and Factors II and X were found most informative, but the abnormal values showed wide individual variations. FDP was demonstrable in nearly all of the sera after treatment. The ethanol-gelation test was positive in 50% of the animals. On the evidence of histological studies, the presence of fibrin was demonstrable in the hepatic, splenic and renal microcirculatory system of the animals thus treated. After endotoxin injection, electroimmunodiffusion revealed a fall in the fibrinogen levels to 50% of the original values.

The results show that any suspicion of DIC calls for the utilization of all practicable laboratory procedures in the interest of correct diagnosis and adequate therapeutic measures.

The clinical problems of haemorrhagic disorders have been centering on disseminated intravascular coagulation (DIC) in recent years. This syndrome of extremely variable manifestations may arise on the basis of diverse primary conditions and seems to account for the majority of coagulation disorders [Lasch, 1970]. The haemostatic defect underlying the condition originates from a diffuse activation of the entire clotting system and gives its clinical and laboratory features a wide, almost individual, diversity which makes diagnosis and therapy likewise difficult.

The aim of the present study has been to produce a model of DIC in its mildest form which, despite of being the most common type of this condition, very often eludes diagnosis because of the scarcity of clinical manifestations. The model was intended for the evaluation of the current laboratory procedures employed in the diagnostics of DIC.

Material and methods

Induction of DIC in rabbits. 14 randomly selected nonpregnant female rabbits of 2.5 to 3 kg body weight were used. 8 rabbits were treated with 100 μg *E. coli* 055; B5 (Boivin) endotoxin per body weight in 5 to 6 ml physiological saline injected intravenously into the ear vein. The other animals serving as controls received physiological saline only.

Immediately before injection the following blood samples were drawn from the ear vein;

1) 1.6 ml blood + 0,4 ml 3.3% sodium citrate;

2) 1 ml blood + 0.05 Trasylol® (Bayer, 20 000 KIU/ml).

The Trasylol® sample of inhibited fibrinolytic activity was left to clot, then, after incubation at 37°C for 2 hours the clot was separated, centrifuged at 2000 r.p.m. for 20 min and the supernatant serum was sucked off. The incoagulable, citrated blood sample was handled in the same manner, however here for all manipulations a silicon-coated system was used.

Sampling was followed by the injection referred to above. 24 hours later samples were taken again and the animals were killed by exsanguination through the femoral artery. Immediately after death, tissue specimens were taken from the liver, spleen, kidney, lung and myocardium for histologic study.

The following studies were carried out.

Blood coagulation studies. The pre-treatment values were compared with those obtained at the end of treatment, the value of the test group being referred to those of the controls.

a) Thrombin time; to 0.1 ml citrated plasma 0.05 ml Owren-buffer [OWREN, 1947] was added and left to clot with 0.05 ml of a thrombin solution 20 NIH-U (= Topostasin® Hoffman la Roche) per ml at 37°C.

b) Thromboplastin time [QUICK, 1935], by the use of a calcium-containing thromboplastin preparation (Thrombokinase, Geigy).

c) Partial thromboplastin time [PROCTOR and RAPPAPORT, 1961] by the use of Inosithin (Associated Conc., New York) as partial thromboplastin.

d) Measurement of fibrinogen [CLAUSS, 1957]. Contrary to the original procedure, only the tenfold plasma dilutions were considered, i.e., estimated for thrombin time. No calibration graph was prepared for the calculations of the fibrinogen concentrations, since the presence of the fibrin monomer and fibrin degradation product (FDP) in DIC interferes with reliable determination of fibrinogen.

e) Ethanol-gelation test for the demonstration of fibrin-monomer [GODAL and ABILDGAARD, 1966].

f) Measurement of prothrombin [KOLLER et al., 1951] by the use of calcium-containing thromboplastic material (Thrombokinase, Geigy). The procedure serves for the determination of "true" prothrombin.

g) Determination of Factor X by the method of HOUGIE [1962,] with the reagents Bentonit (Pro-Labo, Paris), Stypven (Borrough and Welcome, London), Inosithin (Associated Conc., New York).

h) Determination of the number of platelets in Buerker's chamber by the method of FEISSLY and LÜDIN [1949.]

i) Determination of fibrinogen and of fibrinogen degradation product (FDP) by immunoelectrophoresis [JÁKÓ et al., 1970]. The necessary antirabbit fibrinogen serum was raised in goats by means of purified rabbit fibrinogen prepared by the procedure of KAZAL et al. [1963], 2 ml doses of the 1.5% fibrinogen solution having been administered intravenously every second day on 18 occasions altogether, and this being followed by intramuscular immunization with the same fibrinogen doses in complete Freund's adjuvant every four days on 5 occasions altogether. Then, after a pause of 7 days, immunization was continued under antihistamine protection, likewise on five occasions. 15 days after the last injection the animals were killed by exsanguination.

Specificity of the antiserum was checked by immunoelectrophoresis. After exhaustion with rabbit serum (9 : 1) a monospecific antiserum of high titre, giving precipitation exclusively with fibrinogen, was obtained.

Electroimmune diffusion was performed on glass plates measuring 12×8.2 cm in the following system; Agarose (Koch-Light) in 1% Na-Veronal-Veronal buffer of pH 8.6, 8.75 ml; antirabbit fibrinogen goat serum, 3.2 ml; heparin (Chemical Works Gedeon Richter, Budapest), 0.1 ml. The subsequent steps are given in detail in the original description of the procedure [JÁKÓ and SAS, 1970].

Histologic studies. The fresh tissue samples were fixed in 10% formalin and 96% ethanol, embedded in paraffin and stained with Masson—Goldner's trichrome dye [GOLDNER, 1938] which stains the intravasal fibrin precipitates a conspicuous bright orange.

Results were evaluated by Student's *t*-test.

Results

Blood coagulation. The graph in Fig. 1 displays the coagulation data obtained prior to and 24 hours after the injection of endotoxin, the pre-injection values being found at the left, and the post-injection values at the right side of the columns representing the individual tests. The successive columns from left to right represent the platelet count, thrombin time, thromboplastin time, partial thromboplastin time, thrombin time of diluted plasma, the amounts of prothrombin and of factor X, all being expressed in per cent of the values of pooled control plasma. Mathematical analysis revealed significant deviation of the platelet count, partial thromboplastin time, prothrombin and factor X (Fig. 1).

The results of the ethanol gelation test have not been included in Fig. 1. In none of the endotoxin-treated eight animals had been the test positive prior to injection, whereas 24 hours after the administration of endotoxin, positivity of the test was noted in 4 cases, thus indicating the presence of fibrin monomer.

In the controls, i.e., in the animals having been treated with physiological saline instead of endotoxin, the same values were found after as before the administration of endotoxin. Fibrin monomer was not demonstrable in any of the cases.

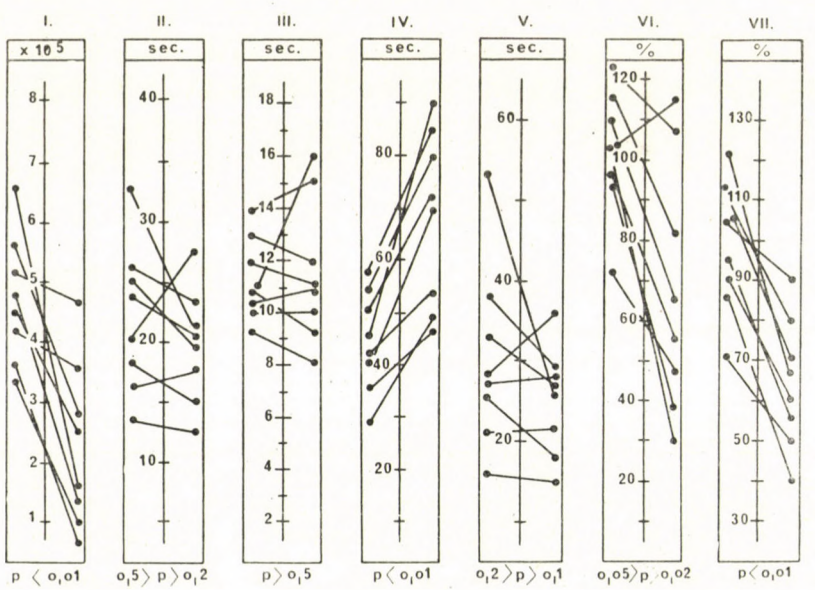

Fig. 1. Results of coagulation studies prior to endotoxin injection (left side of the columns) and 24 hrs after injection (right side of the columns)
I. Platelet count. II. Thrombin time. III. Quick time. IV. Partial thromboplastin time. V. Clauss time. VI. Factor II. VII. Factor X. The individual dots represent the average of two measurements

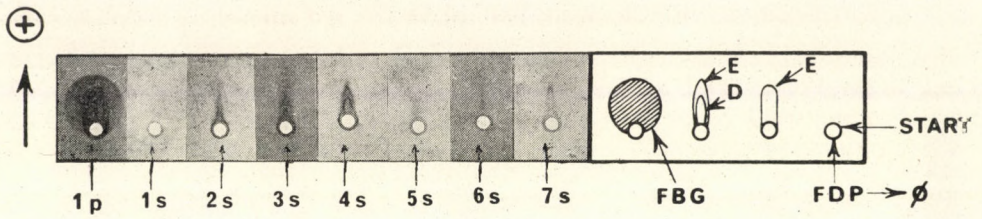

Fig. 2. Results of electroimmunodiffusion studies. 1_p and 1_s: immunograms of plasma and serum before treatment. 2_s—7_s: FDP appearing in the serum after endotoxin administration

The electroimmune diffusion studies revealed mean fibrinogen concentrations of 400 mg per 100 ml rabbit plasma prior to endotoxin injection. 24 hours after injection the fibrinogen concentrations were not higher than 210 mg per 100 ml plasma ($p < 0.02$). Administration of endotoxin thus resulted in a significant fall in the amount of protein marked by the immunological characters of fibrinogen.

FDP was absent from all sera prior to the administration of endotoxin, but was demonstrable in six out or eight animals after injection. Fig. 2 shows the electroimmunograms of the pooled plasmas and sera prior to treatment and of the FDP-containing sera after treatment.

Histology. Apart from quantitative differences, intravascular pre-

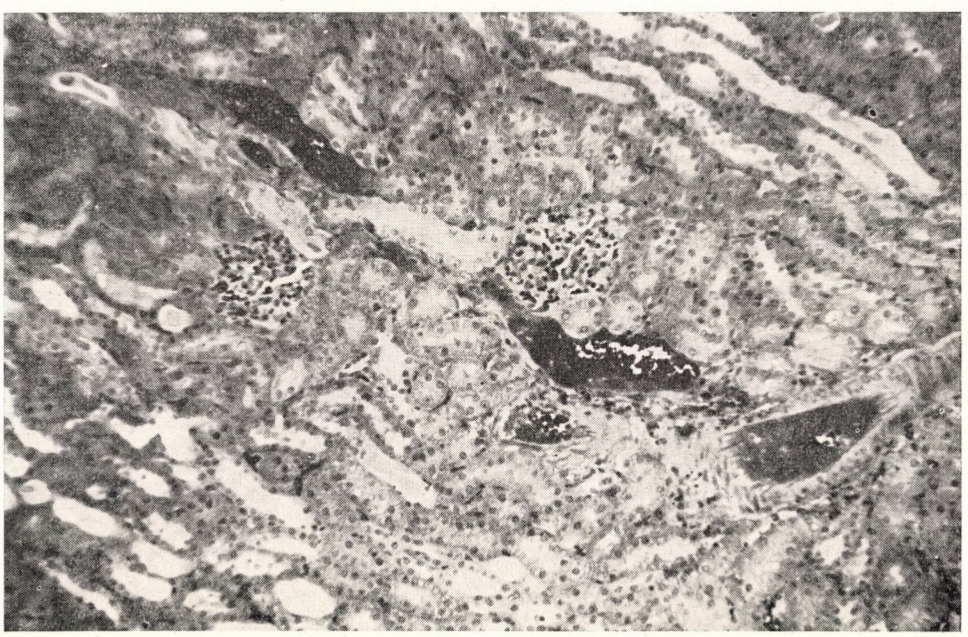

Fig. 3. Clots in the arciform artery (Rabbit kidney. Masson—Goldner staining. ×100)

cipitates were demonstrable in all endotoxin-treated animals, whether fixation in formalin or in ethanol had been used. Of the organs examined, the kidney, spleen and liver exhibited marked intravascular clotting. The precipitates were of variable extent, appearing generally as a homogeneous fibrin mass, though areas of fibrillar structure were also noted. Fig. 3 shows fibrin precipitates in the renal vessels.

Discussion

Our team has been studying for years the diagnostic and therapeutic aspects of DIC [SAS et al., 1968; JÁKÓ and SAS, 1970; SAS et al., 1971; 1971b]. The aim of the present study has been to examine the diagnostic value of the current laboratory tests in the common mild forms of DIC on the grounds of a model experiment. For the induction of DIC, injections of endotoxin or of thromboplastin are usually employed [SELYE, 1966; BELLER, 1971]. The classical procedures are based on the provocation of the generalized Shwartzman phenomenon by two injections of endotoxin separated by a certain interval and giving rise to DIC in its most severe acute form. However, in clinical practice milder forms liable to elude diagnosis are far more common. Therefore, we confined our experiments to the first step of the reaction, to the effect of the preparatory injection of endotoxin, which alone is apt to bring about a generalized activation of the blood clotting system [BELLER, 1971; BLEYL et al., 1969]. It must, however, be emphasized that the clotting and fibrinolytic systems of the laboratory animals greatly differ from those of humans. Therefore, as far as human pathology is concerned, the results of the model experiment should be interpreted with due caution.

It has been found that among all parameters studied, the platelet count, the partial thromboplastin time, factors II and X were most frequently affected. On the evidence of clinical observations [SAS and PÁLOS, 1971], prolongation of the Quick-time and of the partial thromboplastin time as well as a fall in the platelet count are the earliest indicators of DIC.

In all of the endotoxin-treated animals there was a fall in the platelet count. On the other hand, thrombocytopenia was not detectable in more than 60 to 70% of our clinical material. This difference may be due to the thrombocytopenic effect of endotoxin which was hardly ever the responsible factor in our clinical cases. On the other hand, in diffuse coagulation disorders accompanying chronic liver injury, the effect of the toxin may be decisive [JÁKÓ and SAS, 1968; SAS and JÁKÓ, 1968]. Moreover, on confronting the results of animal experiments with those of human observations, it is well to bear in mind that, while in the model experiment DIC has been induced by a single, acute intervention, the clinical condition is prevalently the consequence of a protracted, chronic process.

Though in the model experiment the coagulation disorder has been induced with identical endotoxin doses and under identical experimental conditions, yet the response was nearly as heterogeneous as in the clinical cases. Endotoxin sensitivity of the laboratory animals shows wide individual variations and also depends on sex, age, general condition, etc. Though consideration has been given to all possible factors of this kind, yet the endotoxin-induced condition was by no means uniform. How more heterogeneous must be then the clinical condition where, in addition to all these factors, the primary disease and the responsible factor are also extremely variable.

In certain respects, the immunological procedures are of greater sensitivity than the conventional coagulation tests. However, they require elaborate equipments and are time-consuming. The time factor is not a serious drawback in the presence of chronic forms of DIC, moreover, immunological demonstration of FDP represents here a highly valuable diagnostic clue. However, the discrepancy between the plasma and serum findings which has also been confirmed, raises the possibility of the presence of some artifact [MERSKEY, 1969].

In a number of our clinical cases, even in those of lethal outcome, we have been unable to establish the correct diagnosis of DIC, despite the use of coagulation tests and other diagnostic procedures. On the other hand, in the present model experiment, intravasal fibrin precipitates were demonstrable even in the cases of mildest degree. Histologic study of this kind of the autopsy material in lethal cases would be therefore most instructive by revealing the presence of the diffuse coagulation disorder in retrospect. This might provide useful clues for the assessment of the diagnostic value of laboratory evidence and promote the elaboration of further procedures.

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URINARY EXCRETION OF HYDROXYPROLINE IN PREGNANCY

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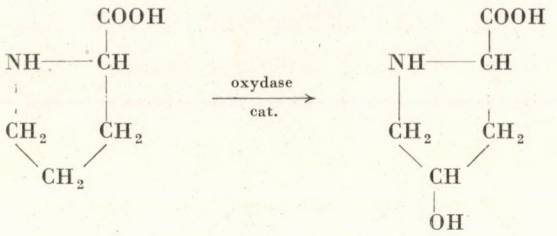
Urinary hydroxyproline excretion has been studied in 10 non-pregnant and 102 pregnant women. Increased values were confined to the second half of pregnancy. Hydroxyproline excretion was similar in imminent abortion as in normal pregnancy, but in cases of premature delivery a significant reduction in the figures was demonstrable.

The metabolism of mesenchymal tissues is significantly increased during pregnancy. This increase is by no means confined to the organs of strictly reproductive function which are under endocrine control but involves other organs as well [12, 13]. The quantitative changes in the fibrous elements and in the ground substance of the uterus have extensively been studied and the mucopolysaccharide content of the ground substance has been found to increase parallel with the enlargement of the pregnant uterus. This increased production of glycosaminoglycan in the tissues seems to account for the high rate of glycoprotein synthesis and for the elevation of the serum glycoprotein level during pregnancy.

Although in pregnancy the total collagen mass of the uterus is significantly increased, the increase in the amount of fibrous components lags behind that of the ground substance. Collagen is known to be the only protein which contains hydroxyproline in large amounts [19, 27]. An increase in the rate of collagen synthesis thus results in an increased production and urinary excretion of hydroxyproline. Conversely, the amount of hydroxyproline excreted in the urine is an index of the rate of collagen synthesis [30, 31, 38]. The composition of this scleroprotein per 100 g protein is as follows.

Alanine	9.5 g
Cystine	0.0
Glycine	27.0
Hydroxylysine	1.5
Proline	15.0
Hydroxyproline	14.0
Valine	3.0

Thus, collagen consists for the greatest part of glycine, proline and hydroxyproline, as indeed the three links of the collagen chain are formed by these



proline ascorbic acid hydroxyproline

Fig. 1. Production of hydroxyproline

amino acids in the sequence, glycine—hydroxyproline—proline. Hydroxyproline is confined to the connective tissue protein of animal origin, and is absent from other proteins.

Hydroxyproline, 4-hydroxypyrrolidine-2-carboxylic acid, is formed from proline by the joint action of an oxydase catalyzer of pluripotential activity and of ascorbic acid (Fig. 1).

Material and methods

The pooled 24-hour urine of 10 non-pregnant and 102 pregnant women in different stages of pregnancy has been studied. Urine was collected during five days, but only the mean of the last three days was considered. In the first two days, collection of urine is often unreliable, therefore it was found preferable to disregard these values. Urinary creatinine was estimated in every case as an index of reliable urine collection. The cases where there was reason to suspect some inaccuracy, were also excluded from analysis. Estimation of hydroxyproline was carried out on the basis of the procedure of STEGEMANN [32] as modified by BERGMAN and LOXLEY [1], and KOEVOET [17].

Reagents

Concentrated HCL,

0,001 M HCL (1 ml concentrated HCl in 1 liter water)

Isopropanol acetate–citrate buffer (PH =6.0) sodium acetate · 3 H_2O, 57 g; trisodium citrate · 2 H_2O, 37.5 g; citric acid · H_2O, 5.5 g;

isopropanol, 385 ml; made up with water to 1 litre.

Oxidant solution: Tosylchloramide sodium 7 g in 100 ml water, made up immediately before use.

Isopropanol.

Ehrlich's reagent (made up immediately before use): p-dimethylamino-benzaldehyde 10 g, dissolved in 11 ml 60% perchloric acid. 3 ml of this solution is added to 16 ml isopropanol before use. Each test tube requires 13 ml of this mixture.

Diluted buffer solution: to 4 parts of the isopropanol-acetate-citrate stock solution 1 part water is added immediately before use.

Diluted oxidant solution: isopropanol-acetate-buffer stock solution and oxidant solution are mixed 4 : 1 before use.

Procedure

1 ml of 24 hr urine is hydrolyzed with 1 ml concentrated HCl in a glas-sstoppered test-tube at 120°C, then rinsed twice with 1 ml 0.001 M HCl into the dish and the contents are evaporated to dryness in a water bath under an exhaust. The dry residue is taken up in 5 ml 0.001 M HCl, transferred into a centrifuge tube and spun for 5 min at 3000 r.p.m. From the supernatant, two 1 ml samples are pipetted into two ground glass-stoppered tubes, 2 ml isopropanol is added to each and shaken. Then 1 ml of the diluted citrate buffer is pipetted into the first and 1 ml of the oxidant solution into the second tube. 13 ml of Ehrlich's reagent is then added to each tube. After shaking, the sealed tubes are kept at 37°C for 17 hours. Both substances are then estimated in a Spektronon 360 photometer at 558 nm against water in 1 ml cuvettes.

Calculation

The difference between the extinction values for the oxidized and the buffered sample permits to read from the calibration curve the amount of hydroxyproline in mg per 100 ml which is multiplied by that of the daily urinary excretion.

The results have been evaluated by Student's *t*-test.

Results

Mean urinary hydroxyproline excretion in 24 hr urines of healthy females between 20 and 30 years of age was 18.7 $\pm$ 2.4 mg. This figure is slightly lower than those given in the literature. ZORAB [39] found 27.1 $\pm$ $\pm$ 1.8 mg in 17 to 18-year-old girls, PLATT et al. [28] 21 $\pm$ 6.2 only. The cause of this inconsistency must be sought in differences in the techniques.

Under normal conditions, the urinary excretion of hydroxyproline is significantly lower in early pregnancy than in non-pregnant women. On the other hand, from the 24th week of pregnancy onward it rises to significantly higher levels than in non-pregnant women and reaches its peak after the 37th week, i.e., close to term, as indeed in this study the maximum figure of 80 mg/24 hr was found in this period.

Urinary hydroxyproline excretion has further been examined in cases of imminent abortion and premature delivery. It was only in the latter group where a significant deviation of the figures from those of normal pregnancy was demonstrable (Table I).

Table I

Urinary excretion of hydroxyproline in women with normal pregnancy, imminent abortion and imminent premature delivery

	Week of pregnancy				
	−12	13−20	21−28	29−36	37−
Normal pregnancy	9.4+1.15* (10)	14.7+1.17 (11)	25.9+3.58 (11)	31.9+2.08 (10)	44.2+2.31 (17)
Imminent abortion and premature delivery	7.8+1.36 (10)	14.2+1.21 (10)	19.4+1.33 (12)	21.9+1.75** (11)	—

(The figures in brackets represent the number of cases)
* mg/24 hr $\pm$ SD
** $p < 0.05$

Discussion

It has been pointed out by MEILMAN et al. [20] and KLEIN and YEN [15] that urinary hydroxyproline excretion significantly declines in the puerperium, an observation implicating that from the collagen degraded in the post-partum period practically no hydroxyproline is excreted into the urine, despite the fact that, as demonstrated by MORRIONE and SEIFTER [22], absorption of collagen in the first days of puerperium as a result of uterine involution amounts to 53 g.

KLEIN and YEN [15] studied urinary hydroxyproline excretion from the 20th week of pregnancy until the 8th week post partum in 6 cases. As in the present study, excretion was found to increase with advancing pregnancy, an observation pointing to an increasing rate of collagen synthesis parallel with the enlargement of the pregnant uterus. On the evidence of animal experiments, the collagen content of the uterine horns is closely related to the size of the uterus and to the number of foetuses [7].

An increase in the amount of uterine collagen toward the end of pregnancy has been noted by several authors. According to animal experiments and human studies, the pregnant uterus is the site of enhanced collagen synthesis. According to CRETIUS et al. [4], the body and cervix of the pregnant uterus are richer in saline-soluble collagen than those of the non-pregnant uterus. This collagen of ready solubility is typical of young connective tissue. On the evidence of the studies by KÜHN with ^{14}C-labelled glycine [18], the saline-soluble collagen is directly transformed into its insoluble definite form. In other words, the young collagen of ready solubility gradually assumes its insoluble mature form.

Though the total collagen of the uterus reaches 6 to 10fold its original value by the end of pregnancy, its concentrations referred to dry substance actually decline to one third [2, 3, 21, 35, 36]. The results of animal experiments are consistent with these findings [3, 7, 9, 24]. The fall in collagen concentration is clearly due to the excessive increase in the bulk of ground substance which is out of proportion to that of the fibrous elements.

It is only the increase of the ground substance made up of proteins and polysaccharides which keeps pace with the growth of the pregnant uterus. At the end of pregnancy the ratio between ground substance and fibrous elements corresponds to 3 : 1 in the uterine body, and to 5 : 1 in the cervix, as against 1.5 : 1 and 2 : 1, respectively, in the non-pregnant uterus. Thus, the total mass and concentration of the amorphous interstitial substance is far in excess of that of the collagen in both uterine body and cervix [4, 33].

Other data also indicate that collagen synthesis is enhanced in the second half of pregnancy. There are, for instance, the studies by HALME and JÄÄSKELÄINEN [6] on the proline hydroxylase activity of protocollagen in

the pregnant mouse uterus. Protocollagen is a collagen precursor consisting of polypeptides of high proline and low hydroxyproline contents in which the proline group is converted to hydroxyproline by the said enzyme. It has been demonstrated by these authors [6] in mice that, while in the first half of pregnancy the activity of the enzyme is the same as in non-pregnants, in the second half it reaches approximately fourfold values. An increased protocollagen proline hydroxylase activity has been noted in the pregnant uterus by KAO et al. [11] too, furthermore it has been demonstrated that oestrogen and progesterone, particularly the former, are activators of the enzyme in question.

In the light of the present findings which are in line with published evidence, the increase in urinary hydroxyproline excretion in the second half of pregnancy may thus be connected with an increased rate of uterine collagen synthesis. This, however, is by no means incompatible with an additional role of the foetus and of the appendages of the ovum, the less so as there is evidence suggestive of this possibility.

As pointed out by NEUMAN and LOGAN [26], hydroxyproline is absent from egg-white as well as from egg-yolk. Its formation in the foetus and its membranes starts during embryonal life. NEUMAN in a later study [26] then showed that hydroxyproline attains demonstrable amounts in the four-day chick embryo and continues to rise to 1.16% of the dry material by the 19th day.

HARKNESS and HARKNESS [8] found that the rate of growth of collagen in the rat foetus is far in excess of that of body weight, the collagen concentrations attaining sixfold values between the 13th and 21st day of intrauterine life. This is also valid for the placenta, umbilical chord, and membranes.

Data on the hydroxyproline excretion of the human foetus are scarce. YOUNOSZAI and HAWORT [37] found low excretion figures during the first three neonatal days in full-term or premature newborns as well as in foetuses with prenatal dystrophy.

Placental hydroxyproline production seems to continue until the end of pregnancy. SCHEUNER and RUCKHÄBERLE [29] found a steady increase in placental hydroxyproline from the sixth week onward until term. The findings of KNOPP [16] also point to a progressive collagenization of the placenta throughout pregnancy.

These views are, however, not universally shared. According to ZHUK development of the connective tissue of the villi is complete before the end of the fourth month and, according to NAGY [25], there is even a regression of placental tissue between the seventh and ninth months.

The present observation that hydroxyproline excretion is significantly less in women with imminent premature delivery than in normal pregnancy

is thus by no means incompatible with an abnormal collagen turnover in the uterus as well as in the foetoplacental system under these conditions.

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IDIOPATHIC THROMBOCYTOPENIC PURPURA: EXAMINATION OF CELLULAR IMMUNITY WITH HOMOLOGOUS PLATELET ANTIGEN IN VITRO

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Lymphocyte transformation, as a cell-mediated immune reaction to specific (platelet) and non-specific (phyto-haemagglutinin) stimulants has been studied in the lymphocyte cultures of 12 patients suffering from idiopathic thrombocytopenic purpura (ITP) and in that of 10 normal subjects. The platelet antigen induced lymphocyte transformation in a large number of the patients but not in the normal subjects. This is indicative of the cellular immunity which plays a role in the pathomechanism of ITP. The method seems to be suitable for diagnostic use.

Introduction

In recent years, the lymphocyte transformation test has increasingly been used for diagnostic and differential-diagnostic purposes chiefly in allergic and autoaggressive conditions [Hirschhorn, et al., 1963; Nowell, 1960; Schellekens and Eijsvoogel, 1968].

The importance of a lymphocyte culture lies in the fact that lymphocyte transformation is an in vitro model of cell-mediated immune reactions. In response to phytohaemagglutinin (PHA), a considerable increase in metabolism begins in the culture's small lymphocytes which synthetize DNA and which, or at least some of which, transform into large basophilic blastoid cells containing nucleoli. Similarly to PHA, other non-specific stimulators of lymphocyte transformation are pokeweed, isolated from Phytolacca americana [Farnes et al., 1964], S-streptolysin [Hirschhorn et al., 1964], staphylococcus alpha-toxin [Ling et al., 1965] and mercuric chloride [Schöepf et al., 1967]. To the group of specific mitogens belong several antigenic or haptenic substances, such as tuberculin [Cowling et al., 1963; Oppenheim et al., 1965; Permain et al., 1963; Schrek, 1963], penicillin [Holland and Maner, 1964; Sarkany, 1967], the endotoxins of Gram negative bacteria [Oppenheim and Perry, 1965], and tissue antigens [Dausset and Rapaport, 1966; Oppenheim, 1969]. While in response to a non-specific mitogen, 30 – 90% lymphocyte transformation can be observed, specific mitogens will only elicit one of 4 – 20%.

It is more than 20 years ago that in ITP the presence of a humoral antiplatelet factor was demonstrated [Harrington et al., 1951]. Later ex-

periments identified this antiplatelet factor as immunoglobulin [SHULMANN et al., 1965]. KARPATKIN found antiplatelet antibodies in 73% of ITP patients [KARPATKIN and SISKIND, 1969]. In recent years several authors have made attempts at applying immunosuppressive treatment in ITP patients. The authors agree in that steroids ensure remission in a large number of cases but the thrombocytopenia returns after treatment has been discontinued [CORTALLARO et al., 1970; MARMONT and FUSCO, 1958]. SUSSMAN et al. obtained good results with azathioprine [1967] and LAROS and PENNER [1971] achieved remission with cyclophosphamide in ITP patients who were resistant to other treatments. Recently, there have been accounts in the literature of cellular immunity in ITP. PIESSENS et al. [1970] elicited lymphocyte transformation with autologous thrombocytes in an ITP patient. According to CLANCY [1972], stimulation with platelets was followed by lymphocyte transformation in six of seven ITP patients. ITP may be regarded as an autoimmune disease in which the presence of auto-antibodies can be demonstrated with serological methods. This gave rise to the question how the cell-mediated type of immunity was implicated in the pathomechanism of ITP, and whether this special type of immunity could be followed with the help of the cell-mediated reaction in vitro.

Material and methods

Examinations were made in 12 patients suffering from ITP and in 10 haematologically and immunologically normal subjects. Lymphocyte transformation was observed in six-day cultures of peripheral leucocytes grown according to a modified method of MOORHEAD et al. [1960].

Isolation of the cells was done by sedimentation: 80—90% of the cellular elements were lymphocytes. The lymphocytes were cultured in a 10-ml centrifuge tube; 1 part of autologous serum inactivated at 56°C was added to 3 parts of Parker's 199 medium and a plasma rich in lymphocytes obtained from heparinized blood was introduced. The number of cells was 10^6/ml, i.e., one culture tube contained 4×10^6 cells. Since in ITP antiplatelet antibodies are present, it seemed obvious to use platelets or platelet extracts as specific antigens.

The thrombocyte antigen was prepared by the method of DAUSSET and COLOMBANI [1964] from a washed platelet suspension obtained from fresh heparinized blood. To each of the cultures 0.05 ml of an aqueous solution of the platelet extract (which contained 0.02 mg dry material), was added. The culture contained 10^6 cells per ml in each case. In addition, for each patient one antigen-free (negative), and one PHA-stimulated (positive) control culture was set up. The cultures of the PHA controls contained 0.08 ml of phytohaemagglutinin P (Difco). These cultures were grown for 72 hrs, while those containing a specific antigen, for six days. Evaluation of each culture was carried out on 1000 cells under the light microscope. The cell forms known as "transitional" were not included into the group of large lymphoblastic cells. Evaluation of lymphocyte transformation was done by two examiners working independently. On the sixth day, another cell count was done and the cells were fixed in the test tube. Before and after surgery the examinations were carried out twice in the ITP patients. Mean results of the preoperative and postoperative examinations are given in Table I. In the control group, too, two examinations were performed. The patients, none of whom blood transfusion had previously been given, now received a transfusion of 360 ml of whole blood only during surgery and in the course of determining platelet life span each of them was given a tagged homologous thrombocyte suspension for diagnostic purposes on one occasion.

Table I

Lymphocyte transformation (per cent) elicited with PHA and thrombocyte antigen, before and after splenectomy

Name	Age, years	Duration of disease, years	Sex	PHA stimulation		Thr. antigen stimulation		Control (without stimulation)	
				before splenectomy	after splenectomy	before splenectomy	after splenectomy	before splenectomy	after splenectomy
1 M. L.	40	1	M	64 (63) (67)	68 (71) (65)	18+ (17.2) (15.2)	14.1++ (15.2) (13.0)	0.0 (0.0) (0.0)	0.2 (0.3) (0.0)
2 T. M.	50	3	F	71 (68) (73)	72 (77) (68)	6.2+ (6.4) (6.0)	7.2+ (8.4) (6.0)	0.2 (0.3) (0.1)	0.1 (0.2) (0.0)
3 B. L.	25	2	F	45 (45) (47)	56 (49) (62)	13.8+ (13.0) (14.6)	11.6+ (10.6) (12.6)	0.0 (0.0) (0.1)	0.0 (0.0) (0.1)
4 Gy. K.	67	2	F	48 (49) (45)	60 (59) (60)	15.0+ (13.6) (16.4)	16.8+ (18.4) (15.2)	0.2 (0.3) (0.0)	0.3 (0.4) (0.3)
5 K. A.	34	2	F	66 (66) (69)	64 (62) (67)	1.6− (1.9) (1.3)	0.8− (0.5) (1.1)	0.3 (0.2) (0.4)	0.2 (0.3) (0.1)
6 Gy. M.	22	2	F	61 (58) (65)	79 (83) (76)	5.8+ (5.4) (6.2)	2.1− (2.3) (1.9)	0.0 (0.0) (0.0)	0.1 (0.3) (0.0)
7 H. M.	32	1	F	31 (25) (39)	45 (40) (49)	1.2− (1.3) (1.1)	0.5− (0.8) (0.2)	0.2 (0.0) (0.4)	0.0 (0.0) (0.1)
8 D. I.	33	3	F	68 (70) (65)	67 (60) (73)	12.6+ (13.0) (12.2)	10.2+ (8.6) (11.8)	0.0 (0.0) (0.4)	0.0 (0.0) (0.1)
9 R. M.	15	2	M	34 (31) (37)	39 (36) (43)	0.6− (0.2) (1.0)	1.9− (2.0) (1.8)	1.1 (0.6) (1.5)	0.2 (0.3) (0.1)
10 V. A.	16	2	M	82 (89) (75)	73 (80) (65)	14.8+ (15.2) (13.4)	18.4+ (20.4) (16.4)	0.2 (0.2) (0.2)	0.2 (0.4) (0.1)
11 B. D.	34	1	M	73 (70) (73)	61 (60) (63)	20.4+ (18.4) (22.4)	12.1+ (9.6) (14.6)	0.4 (0.4) (0.3)	0.1 (0.1) (0.2)
12 Sz. K.	41	2	F	46 (48) (44)	64 (52) (76)	12.8+ (13.6) (12.0)	10.3+ (11.0) (9.6)	0.1 (0.2) (0.1)	0.2 (0.3) (0.2)

In brackets: results of two separate examinations.

Results

PHA stimulation invariably produced adequate, pronounced transformation in ITP lymphocytes (Table I). In response to platelet antigen in three cases (Nos 5, 7 and 9) the transformation value obtained was so low that it could not be regarded as positive. Clinically, each of these three cases corresponded to ITP and this was confirmed by the splenectomy and histological examination excluding other splenic diseases. The remaining nine cases showed definite lymphocyte transformation with platelet antigen. The specific stimulus elicited a low, at most 20.4% transformation. All cases were reexamined nearly a year after splenectomy without finding a significant difference between the preoperative and postoperative value. In order to check whether a platelet extract as a specific antigen elicited lymphocyte transformation in patients not suffering from ITP, cultures were set up with peripheral lymphocytes from 10 patients free from any internal disease. Table II shows that in response to a platelet antigen the rate of lymphocyte transformation never exceeded 2%. (The platelet extract had been obtained from normal subjects and was of the mixed pooled type.)

Table II

Lymphocyte transformation (per cent) elicited with PHA and thrombocyte antigen in healthy subjects

Name	Age, years	Sex	PHA stimulation	Thr. antigen stimulation	Control (without stimulation)
1 N. J.	42	M	72 (80) (64)	0.8 (0.6) (1.0)	0.2 (0.3) (0.2)
2 S. K.	21	F	61 (56) (62)	0.4 (0.7) (0.3)	0.3 (0.4) (0.2)
3 P. J.	19	F	83 (78) (88)	1.1 (0.4) (1.7)	0.4 (0.6) (0.3)
4 B. M.	53	M	54 (66) (42)	0.0 (0.1) (0.0)	0.2 (0.2) (0.3)
5 F. K.	45	M	59 (46) (72)	0.6 (0.5) (0.6)	0.0 (0.0) (0.1)
6 K. M.	49	F	67 (76) (58)	0.3 (0.4) (0.2)	0.0 (0.0) (0.0)
7 A. E.	58	F	47 (54) (40)	0.2 (0.3) (0.1)	0.2 (0.3) (0.1)
8 B. I.	36	F	55 (52) (58)	1.3 (1.7) (0.1)	0.5 (0.6) (0.4)
9 Gy. A.	31	F	73 (66) (80)	0.1 (0.2) (0.1)	0.2 (0.0) (0.4)
10 S. P.	27	M	49 (42) (56)	0.2 (0.0) (0.2)	0.0 (0.0) (0.1)

In brackets: results of two separate examinations.

Discussion

The results showed that in clinical ITP patients lymphocyte transformation could be elicited with a homologous platelet antigen, while in normal subjects this in vitro reaction could not be elicited with the same antigen. Parallel with the examination of cellular immunity, humoral antibody studies were also carried out in each patient, viz. the complement fixation reaction of platelets and the LE serological reaction. The former was negative in all patients, while the latter was positive in two. These results are in agreement with those reported by PIESSENS et al. [1970] and CLANCY [1972]. There was no essential difference in the cellular immune reactions done before and after splenectomy. Surgery, however, resulted in an essential improvement in the clinical condition of every patient. All these seemed to confirm the immune character of ITP. The unimportant deviations from the preoperative values for lymphocyte transformation, as observed in the splenectomized patient, indicate that the clinical improvement manifests itself not in the process itself but in the reduction of the subsequent increased sequestration.

In conclusion, examination of cellular immunity by means of lymphocyte transformation may furnish useful data in the diagnosis of ITP.

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EFFECT ON STEROID METABOLISM OF STC-407 AND CYPROTERONE ACETATE IN FEMALE HIRSUTISM

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1. The amount of testosterone precursors which are determinative of the plasma testosterone level, have been found to diminish in response to the administration of STC-407, as indicated by a significant fall in the urinary excretion of the majority of ketosteroid fractions.

2. There is a shift in the ratio between the plasma DEA-S and A-S levels. The fact that the latter belongs to the metabolites of the former, raises the possibility that STC-407 may have a direct enzymatic point of attack, in addition to its presumed hypothalamic site of action.

3. Cyproterone acetate fails to affect the excretion of the majority of ketosteroid fractions. Its clinical efficacy thus seems to result from a competitive mechanism involving the receptors.

In the pathogenesis of hirsutism a role is played by the plasma androgen hormone level as well as the sensitivity of the receptors. As regards the qualitative and quantitative relationship of these hormones, the plasma testosterone level in healthy women are derived from three sources: 1) testosterone of *a)* adrenocortical (70 to 75%) and *b)* ovarian (25 to 30%) origin; 2) interconversional transformation of androstenedione, likewise of adrenocortical origin and of the other "prehormones" of the tissues, in particular of dehydroepiandrosterone (DEA), etiocholanolone (E), androsterone (A) [2]. The carrier of intracellular activity is a plasma-testosterone derivative, dihydrotestosterone (DHT) which, after its production, may find its way back from the cells to the plasma and from there to other organs where it may also exert a hormonal activity. The daily metabolic clearance of this substance (as far as its irreversibly metabolized ratio is concerned) amounts to approximately 75 μg [10]. Follow-up of the fate of radioactive steroids in the organism has shown that previously formed testosterone accounts for not more than 15% of DHT [2]. This gives the prehormones a primary significance, as indicated by the fact that the greatest part of the circulating testosterone remains inactive by its binding to TeBG, and only its free and albumin-bound fractions are involved in metabolism [1], as opposed to androstenedione which is not bound to TeBG and finds therefore free access to the interior of the cell. In females with hirsutism the metabolic clearance of testosterone is far in excess of that of healthy women, it being nearly of the same rate as in males. This is the reason why under certain abnormal conditions, despite

Table I

Plasma cortisol levels and urinary KS-fractions under

Patient	Time of study	Normal scatter:	Plasma cort. 16 ± 10 µg/100ml	HOE 0.1–0.5	HOA 0.2–0.6	OE 0.7–1.4
V. K.	I		8.8	0.2	0.8	0.4
	II		10.0	0.2	0.2	—
	III		13.4	0.1	0.1	—
	IV		6.4	0.7	1.0	0.8
	V		17.2	—	0.9	0.4
E. Gy.	I		12.0	0.8	0.7	1.0
	II		7.8	0.3	0.3	0.6
	IV		4.6	0.4	0.3	0.5
	V		8.6	1.0	0.3	1.1
	VI		—	—	0.8	0.3
N. J.	I		9.0	0.2	0.3	0.2
	II		13.2	—	0.1	—
	IV		12.6	—	0.1	0.1
	V		25.8	—	0.4	0.3
	VI		9.2	—	0.5	0.3
P. J.	I		12.2	0.2	0.9	0.4
	II		12.5	—	0.2	—
	IV		13.0	—	0.3	0.2
	V		13.2	—	0.7	0.3
	VI		21.8	—	0.8	0.2
K. I.	I		34.5	0.6	0.6	0.6
	II		17.2	—	0.1	—
	III		12.6	0.2	0.2	0.3
	IV		10.4	0.3	0.6	0.4
	V		18.8	1.1	0.8	0.3
	VI		17.2	—	0.8	0.2
H. M.	I		8.8	0.5	0.4	0.5
	II		—	0.3	0.1	0.4
Sz. M. Zs.	I		10.0	0.5	0.4	0.4
	II		8.4	—	0.1	—
	III		16.4	0.1	0.2	0.1
	IV		11.2	0.1	0.2	0.1
	V		6.4	—	0.6	0.3
	VI		10.6	—	0.7	0.2

I = STC-407 basic values; II = STC-407 at 30 days; III = STC-407 at 60 days; IV = after second 10-day course of cyproterone

HOE = 11-OH-etiocholanolone
HOA = 11-OH-androsterone
OE = 11-keto-etiocholanolone
DEA = dehydroepiandrosterone
E = etiocholanolone

The influence of STC-407 and cyproterone acetate mg/100ml

DEA 1.05—2.4	E 1.2—4.3	A 1.5—4.1	THF 0 5—2.0	ATHF 0.1—1.2	THE 1.1—5.5	11-D 0.0—1.5
0.6	0.9	2.1	0.4	0.1	1.2	—
—	1.2	1.9	—	—	0.6	—
—	0.6	0.8	—	—	0.6	0.1
1.2	2.4	3.2	0.3	0.1	2.4	—
1.4	1.9	3.2	—	0.4	1.9	—
2.3	4.7	2.8	0.6	0.2	2.9	—
—	0.6	0.3	0.2	—	0.1	0.4
—	3.0	1.6	0.8	0.4	3.2	0.5
0.5	2.7	2.6	0.8	0.3	2.6	—
0.2	1.1	1.3	—	0.1	2.3	0.2
—	0.7	2.1	0.5	0.2	1.4	—
—	0.3	0.9	—	—	0.3	0.3
—	0.6	1.4	0.3	0.2	1.3	0.1
0.2	0.6	1.5	—	0.2	1.4	0.2
—	0.4	1.2	—	—	1.3	0.3
0.6	1.7	2.3	0.2	—	1.5	—
—	0.8	1.1	—	0.1	0.7	0.5
—	1.0	1.5	0.5	0.2	1.8	0.1
0.3	1.0	1.8	—	0.5	2.0	0.1
—	0.9	1.6	—	0.5	2.3	0.4
0.5	1.6	2.2	0.2	—	1.2	—
—	0.6	0.5	0.2	—	0.2	—
—	0.8	0.8	0.2	—	0.4	—
—	2.1	2.6	0.5	0.3	1.8	—
0.3	1.1	2.1	—	0.2	1.3	—
—	1.0	2.5	—	—	1.6	0.3
0.6	2.1	1.3	0.3	0.3	0.9	—
0.4	0.7	0.7	0.3	0.3	0.7	0.4
—	0.9	1.8	0.5	0.6	1.6	0.1
—	0.2	0.2	0.1	—	0.2	—
—	0.8	0.4	0.1	—	0.3	—
0.5	1.0	1.2	0.2	0.1	1.1	—
—	1.0	1.6	—	0.1	1.4	—
—	0.8	1.6	—	0.2	1.2	0.1

10 days after completion of treatment; V = after first 10-day course of cyproterone; VI =

A = androsterone
THF = tetrahydrocortisol
ATHF = allo-tetrahydrocortisol
THE = tetrahydrocortisone
11-D = 11-deoxy-17-OH-corticoids

entirely normal total plasma-testosterone levels, the hair follicles, even though their specific sensitivity or enzymatic supplies should be normal, receive abnormally high amounts of hormone, a constellation which might be compared to the clinical consequences of the accelerated breakdown of thyroxin of shortened half-life [7]. The role of the peripheral receptors pointed out above is of obvious importance in local (non-virilizing) hair-growth. There may be also differences in the disappearance of the hormone. While in women of normal constitution degradation of testosterone takes place almost in its totality in the liver, in hirsutism the hormone is utilized to 32% outside the liver and thus made available to the hair follicles. In view of the fact that androstenedione accounts for 50% and DEA for 20% of circulating testosterone [2], it was deemed of interest to examine the effect of STC-407 (6-dehydro-16-methylene-hydrocortisone) and of cyproterone acetate (6-chloro-17-hydroxy-1-alpha, 2-alpha-methylenepregna-4,6-dienne-3,20-dione-acetate) on the plasma level of DEA-S and of androsterone-S and on the urinary excretion of the 17-ketosteroid and 17 OH-corticoid fractions, the first substance being known to influence adrenocortical hormone secretion, and the second to oppose a competitive mechanism to the androgens at the level of the target organs.

Material and methods

Seven female inpatients having sought medical advice for hirsutism were studied On the evidence of endocrine studies, the abnormal hair growth could be identified as "simple' hirsutism in five, and in particular of the abnormalities of sugar metabolism as well as of the features of the circadian rhythm as hypertrichosis accompanying cushingoid obesity in two cases. The results of the studies made this classification unnecessary. The cases in question were qualified as "simple" hirsutism also on the evidence of the finding that the basic ketosteroid fractions remained within the normal scatter all throughout (Table I).

The patients had first continuous STC-407 treatment in doses of 30 mg daily for 60 days. The parameters under study were determined at the start of treatment, subsequently at 30 and 60 days of treatment and finally, 8 to 10 days after its completion, by the method of FEHÉR et al. [4, 5]. Then cyproterone acetate was given for the first 10 postmenstrual days in two doses of 10 mg daily. A similar course of cyproterone was given after the next menstruation. The patients were advised against possible pregnancies during treatment in view of the hazard of intrauterine feminization of male foetuses by cyproterone [6]. Consideration was given to possible changes of the plasma cortisol level in the course of treatment.

Results

The clinical response to treatment (decrease of hirsutism) and the occasional secondary effects (minor variations of body-weight, lenghtening of the menstrual period) have been dealt with elsewhere [8]. Here we give only the data relative to the levels and excretion of androgen.

While the plasma DEA-S level diminished in response to STC-407 treatment, that of A-S tended to rise (Table II).

Table II

Plasma dehydroepiandrosterone-S and androsterone-S levels under the influence of STC-407

Patient	Time of study	Norm. value D-S 63 ± 14	A-S 21 ± 9 (μg per 100ml)
V. K.	a)	58.5	9.4
	b)	35.0	17.9
K. I.	a)	104.2	14.5
	b)	61.7	23.8
Sz. M. Zs.	a)	70.0	38.7
	b)	44.7	48.3

a) before treatment
b) after treatment

With the exception of OE, all the urinary ketosteroid fractions studied diminished in the course of the study, the majority (HOA, E, A, THF, THE) to the level of significance. The fractions were assessed in every case, for category I in 7, for category II in 7, for category III in 3, for category IV in 6, for category V in 6, for category VI in 5 cases. Symbol "n" in the Tables represents the number of cases suitable for mathematical analysis. When, in consequence of the limits of sensitivity of the procedure, the amount of some individual fraction was too small to be expressed quantitatively, it was excluded from statistical analysis, even though it was sufficient for the demonstration of the suppressive effect of the agent used. After treatment had been discontinued, the majority of the parameters returned to the pre-treatment level by the standards of statistical significance (Tables III, III/a).

Changes in the plasma cortisol level and of the urinary fractions were studied after completion of both courses of cyproterone treatment. In opposition to a temporary rise in individual fractions, etiocholanolone exhibited a significant fall (Tables III, III/a).

Discussion

STC-407 has been found to suppress the function of the hypothalamo-pituitary system in the manner of glycocorticoids [3], thus lowering the ACTH content of the anterior lobe and inhibiting the release of ACTH from rat pituitary cells in vivo as well as in vitro, furthermore reducing the rat plasma and adrenocortical corticosterone levels. On the evidence of our studies it also affects the plasma and urinary levels of the androgen fractions. The

Table III

Mean values of urinary androgenic KS fractions under the influence of STC-407 and of cyproterone acetate mg/100ml

	n	HOE	n	HOA	n	OE	n	DEA	n	E	n	A
I	7	0.43±0.12	7	0.6±0.12	7	0.5±0.2	5	0.66±0.21	7	1.8±0.36	7	2.1±0.12
II	3	0.3	7	0.2±0.01	2	0.5	–	—	7	0.6±0.3	7	0.8±0.5
III	2	0.1	3	0.2	2	0.2	—	—	3	0.7	3	0.7
IV	4	0.4±0.2	6	0.4±0.3	6	0.4±0.3	2	0.85	6	1.7±0.9	6	1.9±0.8
V	2	1.1	6	0.6±0.2	6	0.5±0.3	5	0.5±0.2	6	1.4±0.8	6	2.1±0.7
VI	—	—	5	0.7±0.2	6	0.3±0.1		—	5	0.8±0.3	5	1.6±0.5
Significance	I—II	N.S.	I—II	$p<0.1$	I—IV	N.S.	I—VI	N.S.	I—II	$p<0.05$	I—II	$p=0.001$
	I—III	N.S.	I—III	N.S.	I—V	N.S.			I—IV	N.S.	I—IV	N.S.
	I—IV	N.S.	I—IV	N.S.	I—VI	N.S.			I—V	$p<0.1$	I—V	N.S.
	I—V	—	I—V	N.S.	V—VI	N.S.			II—IV	$p<0.02$	II—IV	$p<0.02$
	I—VI	—	I—VI	N.S.					V—VI	$p<0.1$	V—VI	N.S.
			II—IV	$p<0.01$								
			II—VI	$p<0.05$								
			V—VI	N.S.								

I = STC-407 basic values; II = STC-407 at 30 days; III = STC-407 at 60 days; IV = 10 days after completion of treatment; V = after first 10-day course of cyproterone; VI = after second 10-day course of cyproterone

Table III/a

Mean values of plasma cortisol μg/100ml and of urinary glycocorticoid KS fractions under the influence of STC-407 and of cyproterone acetate mg/100ml

	n	Plasma cortisol	n	THF	n	ATHF	n	THE	n	11-D
I	7	13.9±9.2	7	0.4±0.02	7	0.25±0.05	7	1.5±0.18	—	—
II	6	11.8±3.9	4	0.2±0.1	2	0.2	7	0.4±0.3	3	0.4
III	3	14.1	2	0.3	—	—	—	—	—	—
IV	6	9.7±3.4	6	0.4±0.2	6	0.2±0.1	6	1.9±0.8	2	0.15
V	6	15.0±7.1	—	—	6	0.3±0.2	6	1.8±0.5	2	0.25
VI	4	14.7±5.9	—	—	3	0.3	5	1.7±0.6	5	0.3±0.1
Significant	I—II	N.S.	I—II	$p<0.05$	I—IV	N.S.	I—II	$p<0.01$		
	I—IV	N.S.	I—IV	N.S.	I—V	N.S.	I—IV	N.S.		
	I—V	N.S.	II—IV	$p<0.1$			I—V	$p<0.05$		
	I—VI	N.S.					I—VI	N.S.		
							II—IV	$p<0.001$		
							V—VI	N.S.		

I = STC-407 basic values; II = STC-407 at 30 days; III = STC-407 at 60 days; IV = 10 days after completion of treatment; V = after first 10-day course of cyproterone; VI = after second 10-day course of cyproterone

modification in the interrelationships of the individual fractions are suggestive of a direct action upon the adrenocortical enzyme system. This would seem to account for the favourable clinical effect of the drug on hirsutism [9]. It is, furthermore, possible that at lower glycocorticoid levels, the well-known "permissive effect" on the sensitivity of the androgen receptors is less operative.

In view of the relatively scanty data, all that the modifications of the plasma DEA-S and A-S levels permit to note is their reciprocal tendency.

The for the greatest part statistically significant fall in the urinary excretion of the ketosteroid fractions indicates that the part available for metabolism greatly diminishes in the course of treatment. From this it follows that the antiandrogenic effect of STC-407 is largely due to a reduced secretion of androgens by the adrenal cortex as a result of the suppression of adrenocortical hormone production referred to above.

The practically uniform rise in the values consequent upon the interruption of treatment may be regarded as a spontaneous normalization rather than as an "escape phenomenon" [3], in other words, the enzyme systems of the hormone-producing tissues have suffered no irreversible damage from the agents used. According to BERTHOLD [3], the values start to rise on the 31st day in the case of continuous administration, an observation which we have been unable to confirm under similar conditions.

A transitory, non-significant, initial rise in the plasma cortisol level has been noted in response to cyproterone acetate, while some of the urinary ketosteroid fractions, in particular etiocholanolone among the androgens and THE among the glycocorticid metabolites, exhibited a significant increase. This is at variance with the findings of RAUSCH-STROOMANN [9], according to which this agent of gestagenic properties exerts a suppressive effect on the metabolic clearance of plasma as a result of a competitive mechanism.

After the second 10-day course of cyproterone acetate a further rise was noted in the urinary 11-deoxy-17-OH steroids, in contrast to the 11-deoxy-17-ketosteroids (DEA, E, A) the urinary excretion of which was found to decline. The fact that the 11-deoxy-17-OH fraction contains the precursors of cortisol biosynthesis, lends further support to our view that cyproterone acetate acts directly on the adrenocortical system, in addition to its competitive action on the target organs.

In opposition to some data in the literature, no reduction in the plasma cortisol level was demonstrable in the present study. Considering the small number of cases, we have been unable to form any definite view on the results obtained in the therapy of Cushing's syndrome. On the other hand, after discontinuation of STC-407 treatment we noted a fall in the plasma cortisol level, an observation which is inconsistent with the results of RAUSCH-STROOMANN et al. [9] who found a complete normalisation of the cortisol

level in this "rebound" phase. As expected, 28 out of 33 measurements in this study revealed statistically significant deviations, thus lending support to the interpretation given above.

*

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AN ANOMALOUS LACTATE DEHYDROGENASE ISOENZYME BAND IN ACUTE VIRAL HEPATITIS

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An anomalous fraction, termed "HP-band", was found between the isoenzyme fractions LDH_2 and LDH_3 in a 25-year-old female between the 3rd and 16th day of acute viral hepatitis.

Normalization of the isoenzyme pattern, including disappearance of the extra band, ensued parallel with clinical recovery.

In the light of recent evidence relating to the typical distribution of lactate dehydrogenase (LDH) and of its five isoenzyme fractions in diseases of the heart, liver, malignant tumours and anaemia, estimation of these factors has come to be regarded as an essential diagnostic procedure in clinical medicine [3, 8, 11, 14, 15, 16, 19, 20]. However, the LDH-isoenzyme-distribution may not always be characteristic even in well-defined clinical conditions. KREUTZER et al. [9] reported on irregularities in electrophoretic mobility of the LDH isoenzymes in liver disease. HOENIGOVA and HOENIG [1967], LUBRANO et al. [1971] described an extra LDH band connected with isoenzyme polymorphism in cirrhosis of the liver, LUNDH [1967] and VOIGT [1967] in acute myeloid leukaemia, VOIGT [1967], BIEWENGA and THIJS [1970] in acute myocardial infarction, BUCKELL and BARNES [1968] in glioma, FUJIMOTO et al. [1968] in secondary carcinoma of the liver, and FORBES et al. after repeated blood transfusions [1971]. We have observed an extra LDH-band in a case of acute viral hepatitis. The band appeared between fractions LDH_2 and LDH_3 and proved reversible.

In 1971, the sera of all patients with acute viral hepatitis were submitted to repeated estimation of total LDH [1] and to agar-gel electrophoresis [18] for the study of LDH isoenzymes. In 53 cases, five isoenzyme bands and, as a sign of hepatocellular lesion, a rise in fraction LDH_5 were noted. In the case to be reported an anomalous sixth band was identified between fractions LDH_2 and LDH_3.

Cs. L., a 25-year-old female patient, was admitted on March 31, 1971, with typical signs of acute infectious hepatitis. The diagnosis was confirmed by laboratory tests (Table I).

Tal 1

	Serum						
	Bilirubin in total mg per 100 ml	Bilirubin direct mg per 100 ml	Thymol test Maclagan units	GOT WU	GPT WU	LDH total WU	Alkaline phosphatase
Admission							
March 31	6.4	5.2	6.9	1240	1350	427	—
April 2	—	—	—	—	—	—	—
April 5	—	—	—	—	—	—	—
April 6	8.6	7.2	9.4	320	600	74	—
April 7	—	—	—	—	—	67	
April 9	—	—	—	—	—	62	
April 13	2.4	1.6	8.5	74	72	—	—
April 15	—	—	—	—	—	86	
April 19	1.4	1.3	6.4	22	32	—	6.6
Discharge							
April 22	—	—	—	—	—	42	—
Follow-up							
May 5	0.9	—	2.0	36	16	—	—
Follow-up							
July 7	0.8	—	1.1	11	2	58	—

The condition improved rapidly and so did the laboratory tests. The patient was discharged on April 21, 1971, and then reported for follow-up at our hepatitis clinic.

The smooth clinical course and the favourable outcome of the process were reflected by the laboratory findings (Table I). However, the LDH-isoenzyme spectrum revealed a definite irregularity (Fig. 1). On the third day after admission, the enzymogram showed five bands and, within this spectrum a marked rise in fraction LDH_5, as a sign of hepatocellular damage. Three days later, a sixth fraction of 6% was found between bands II and III; it was termed band HP. In order to ascertain its presence beyond doubt, we repeated the study two days later and found the band more distinct (11%). In the next two days its activity declined (3%) and on the 16th day after admission the abnormal fraction was no longer demonstrable. The enzyme pattern became normalized by the 23rd day after admission, to remain normal subsequently.

						Urine
LDH_1 per cent	LDH_2 per cent	LDH_{HP} per cent	LDH_3 per cent	LDH_4 per cent	LDH_5 per cent	Bilirubin, Urobilinogen
—	—	—	—	—	—	positive increased
31	26	Ø	6	7	30	
43	24	6	15	3	9	
—	—	—	—	—	—	positive increased
25	36	11	14	9	5	
53	22	3	10	6	6	
—	—	—	—	—	—	Ø increased
35	25	Ø	17	16	7	
—	—	—	—	—	—	Ø normal
38	37	Ø	21	3	1	
—	—	—	—	—	—	
35	45	Ø	14	4	2	Ø normal

The possibility that the extra band was due to a dissociation and recombination as a result of successive refrigeration and heating [11] could be ruled out since fresh sera were always used. Sera incubated with NAD-NAD_2 do not give an extra band of the location observed with our fraction [9]. We may thus regard the HP-band in question as a genuine one.

An extra LDH-band of similar location has been described by FUJIMOTO et al. [1968] and WILKINSON [1970] in tumour patients, by BIEWENGA and THIJS [1970] in acute myocardial infarction and by LUBRANO et al. [1971] in cirrhosis of the liver.

The mechanism of production of the irregular LDH-band is unclear. As indicated by the findings of FUJIMOTO et al. [1968] it may well result from a binding between LDH and some pathological serum protein appearing in the course of the disease (hepatocellular necrosis, high thymol values due to labile proteins), and it might represent a hybrid of a normal and of an abnormal LDH "M" subunit.

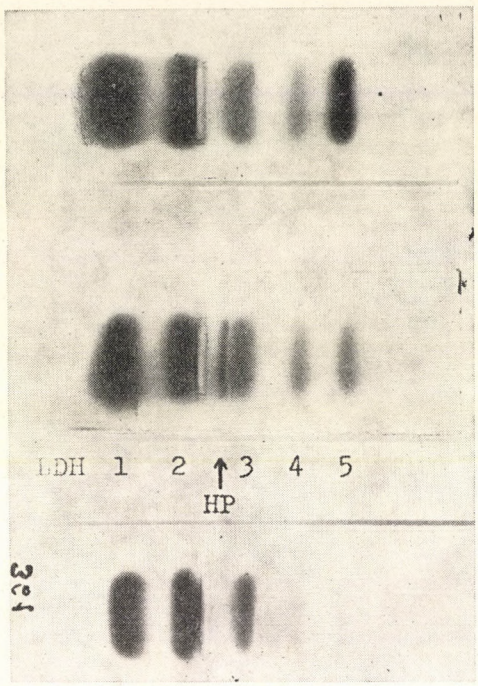

Fig. 1. LDH isoenzymes separated by agar-gel electrophoresis, with anomalous LDH-isoenzyme (HP) band in a case of acute viral hepatitis. Top: increase to 30% of LDH_5 on 3rd day of disease. Centre: anomalous sixth (HP) band between LDH_2 and LDH_3 in serum on 8th day of disease. Bottom: 4 months later, normal LDH-isoenzyme spectrum

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RED EYES IN RENAL DISEASE

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The phenomenon of red eyes due to conjunctival calcium deposition has been observed in four cases of chronic renal disease. Hypercalcaemia with normophosphataemia was present in one, hypocalcaemia with normophosphataemia in two, and normocalcaemia with hyperphosphataemia in one, of these cases. In the last-named case the red eye appeared during intermittent haemodialysis. Histological evidence confirmed the presence of calcium deposits in the basal cells of the conjunctival epithelium. In two cases the mastocyte granules of the conjunctiva also revealed calcium deposits: this points to a possible involvement of mastocalciphylaxis.

On the grounds of published evidence and of the present observations, it is suggested that the red eyes may arise on the basis of various pathomechanisms.

Conjunctival calcium deposition associated with hypercalcaemia was first described by MEESMAN [9], in 1938. This observation was followed by reports on similar cases (HALDIMANN [7]; WALSH [12]). Hypercalcaemic conjunctival deposition was first connected by COGAN et al. [4] with renal disease; this question has, however, received little attention.

The intricacy of the problem is emphasized by recent reports on conjunctival calcium deposition in the presence of hypocalcaemia. BERLYNE and SHAW [2] noted calcium deposits causing local irritation; the condition was termed red eyes. In their 15 cases of chronic renal failure, hyperphosphataemia with a normal or diminished calcium level was found. As it has been pointed out by BERLYNE [3], conjunctival calcium deposition is not invariably associated with hyperaemia; he termed these cases white eyes. There have been sporadic observations of corneal and conjunctival calcification in connection with chronic haemodialysis also [1, 10].

We have noted red eyes due to calcium deposition in four cases; in one with high calcium and normal phosphate, in two with low calcium and normal phosphate, and in one with normal calcium and high phosphate serum levels.

Case reports

Case 1. In L. I., a 18-year-old female patient, arterial hypertension was noted as an incidental finding in November, 1968. She was first admitted in March, 1969. On admission the relevant findings were: blood pressure, 250/190

mmHg; ESR, 42 mm/hour; ASO, 100 U. In serum: complement, 1.2 U; NPN, 56 mg per 100 ml; serum Na, 143 mEq/l; serum K 4.4 mEq/l; serum creatinine, 1.5 mg per 100 ml. Inulin clearance, 20 ml/min; PAH clearance, 107 ml/min; FF, 0.19; urinary protein excretion, 4 g/24 hr. Addis count: 104 million erythrocytes; 12 million leukocytes, 200,000 casts. Dilution-concentration test, 1002 to 1012; urinary bacterial culture, sterile. Infusion pyelography revealed a considerable delay in the excretion of both kidneys which were markedly reduced in size. On the evidence of isotope renography the secretory phase was greatly, and the excretory phase distinctly protracted. The clinical diagnosis was chronic glomerulonephritis.

Azathioprine, 1.5 mg/kg daily was prescribed; it resulted in a fall in protein excretion without affecting haematuria to any significant degree. In September 1969, this treatment was repeated with doses of 3 mg/kg body weight daily. In spite of some diminution of the clinical activity, renal function remained unaffected.

The patient was again admitted in a state of uraemia on July 7, 1970. The red eye developed during this hospitalization.

The essential findings were: blood pressure, 170/110 mmHg; NPN, 98 mg per 100 ml. In serum: creatinine, 16 mg per 100 ml; serum albumin, 4.1 g per 100 ml; serum Ca 14.5 mg per 100 ml; serum P 4.1 mg per 100 ml; serum alkaline phosphatase, 4 BU. Urinary protein excretion, 2.8 g/24 hr; Addis count: 28 million erythrocytes, 2 million leukocytes, no casts.

The conjunctiva of the left eye was injected. Hyperaemia extended from the limbus outward. Slit-lamp examination revealed a thickening and white plaques of the limbic zone as a sign of calcium deposition. The corneal edge appeared as a greyish zone of fine granular structure from which biopsy material was taken.

The further clinical course was marked by progressive deterioration with oliguria and serum creatinine attaining 20 mg per 100 ml. She was transferred to the Dialysis Unit of the Urological Department on September 17, 1970, and died on September 28, 1970. Post-mortem diagnosis was chronic glomerulonephritis.

Case 2. D. H., a 19-year-old female patient. In January, 1964, she noted swelling of eyelids and ankles subsequent to an infection of the upper airways. She was hospitalized and acute glomerulonephritis was diagnosed. She was first admitted to our Department on August 1, 1964. Relevant findings were: blood pressure, 120/80 mmHg; ESR, 24 mm/hr, ASO, 84 U. In serum, complement: 0.2 U; NPN, 21 mg per 100 ml; serum Na, 137 mEq/l; serum K, 5.2 mEq/l; serum creatinine, 0.72 mg per 100 ml. Endogenous creatinine clearance, 48 ml/min. Proteinuria 2 g/24 hr. Addis count: 6 million erythrocytes, 2 million leukocytes, no casts. Renal biopsy confirmed the presence of proliferative glomerulonephritis.

Prednisolone was prescribed in daily doses of 50 mg, subsequently for maintenance treatment, 10 mg daily. After discharge, she reported at the clinic at regular intervals.

When readmitted for the seventh time, in June, 1967, she was practically symptom-free. Essential findings: blood pressure, 150/100 mmHg; NPN, 25 mg per 100 ml; serum creatinine, 1.3 mg per 100 ml; serum complement, 0.3 U; serum albumin, 3 g per 100 ml; serum Ca, 7.4 mg per 100 ml; serum P, 3.2 mg per 100 ml; serum alkaline phosphatase, 4.2 BU. Proteinuria, 1.5 g/ /24 hr; Addis count: 54 million erythrocytes, no leukocytes, no casts.

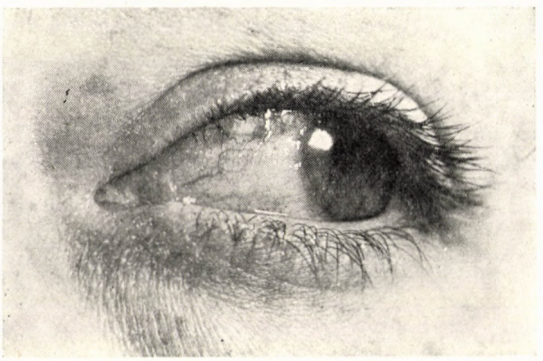

Fig. 1. D. H. Red eye involving the limbus of the left eye

On the tenth day after admission a hyperaemic sector spreading from the limbus of the left eye was noted (Fig. 1); it disappeared spontaneously in a few days.

Case 3. N. I., a 27-year-old female patient, in January, 1970, had experienced swelling of the eyelids in connection with acute tonsillitis. This had been followed by elevation of blood pressure and proteinuria. She had been referred to a hospital where acute glomerulonephritis had been diagnosed. She was first under our treatment as an inpatient between May 22 and September 5, 1970. On admission the essential findings were: blood pressure, 160/110 mmHg; ESR, 48 mm/hr; ASO, 240 U. In serum: complement, 1.2 U; NPN, 107 mg per 100 ml; serum Na, 139 mEq/l; serum K, 4.6 mEq/l; serum creatinine, 5 mg per 100 ml. In urine: protein, 2.5 g/24 hr; Addis count: 38 million erythrocytes, 17 million leukocytes, 200,000 casts.

In an attempt to halt the rapidly progressing course of the process, azathioprine was given in doses of 3 mg/kg body weight daily. As a result, haematuria and azotaemia diminished, but the treatment had to be discontinued because of leukopenia. On her next admission, azathioprine was attempted for the second time. Its effect was favourable but induced leukopenia.

The patient was admitted for the third time in April, 1971. The essential findings were: blood pressure, 200/120 mmHg. In serum: NPN, 81 mg per 100 ml; serum creatinine, 5.7 mg per 100 ml; serum albumin, 4.1 g per 100 ml; serum Ca, 7.6 mg per 100 ml; serum P, 4.4 mg per 100 ml; serum alkaline phosphatase, 1 BU. Urinary protein excretion, 1.3 g/24 hr. Addis count: 90 million erythrocytes, 8 million leukocytes, no casts.

On the third day after admission hyperaemia of the nasal part of the conjunctiva was noted. Slit-lamp examination revealed white granular deposits in this area. Conjunctival biopsy was performed.

The patient was admitted for the last time in October, 1971. Her condition was deteriorating, therefore she was transferred to the Dialysis Unit of the Department of Urology where she died on February 5, 1972. The post-mortem finding was chronic glomerulonephritis.

Case 4. N. I., a 47-year-old male patient had been treated for high blood pressure since 1955. Early in 1971 he had noted gross haematuria subsequent to an acute tonsillitis. From this time onward he went steadily downhill. Arterial hypertension became fixed and he was uraemic when admitted on September 17, 1971.

On physical examination the heart was enlarged to the left by 1.5 cm. There was tachycardia and an accentuated 2nd aortic sound. The relevant findings were: blood pressure, 270/190 mmHg; ESR, 25 mm/h; ASO, 336 U. In serum: complement, 1 U; NPN, 66 mg per 100 ml; serum Na, 133 mEq/l; serum K, 3.0 mEq/l; serum creatinine, 6.2 mg per 100 ml. Urinary protein excretion, 2.9 g/24 hr. Addis count: 2 million erythrocytes, 2 million leukocytes, no casts.

The process remained unaffected by symptomatic treatment, and the patient was started on intermittent haemodialysis. After the fifth haemodialysis conjunctival hyperaemia appeared on the lateral area of both eyes. Biopsy material was taken from the conjunctiva of the left eye.

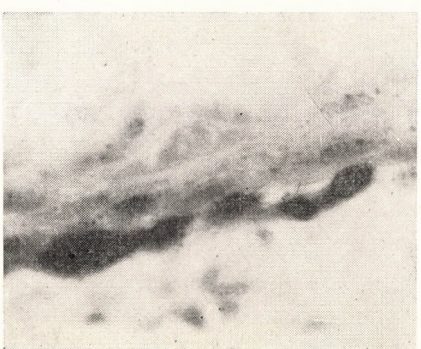

Fig. 2. L. I. Calcium deposits in the basal cells of the conjunctiva. Kossa's method. ×400

Fig. 3. N. I. Calcium deposits among the collagen fibres of the cornea. Kossa's method. ×400

The essential findings at this time were: blood pressure, 200/140 mmHg; NPN, 121 mg per 100 ml. Serum creatinine, 7.1 mg per 100 ml; serum albumin, 3.2 g per 100 ml; serum Ca, 11 mg per 100 ml; serum P, 10.2 mg per 100 ml; serum alkaline phosphatase, 2.1 BU.

The patient died on October 24, 1971. The clinical diagnosis of malignant hypertension was confirmed at necropsy.

Histology of conjunctiva and cornea. Biopsy was performed in Cases 1, 3 and 4. In the histological sections, calcium was revealed by the method of KOSSA. The site of deposition was the basal cells of the epithelial layer of the conjunctiva (Fig. 2). This is consistent with the reported observations confirmed histologically. In the fourth case, examination of the cornea revealed calcium deposits among the collagen fibres (Fig. 3). The mast cell granules also contained calcium deposits (Fig. 4).

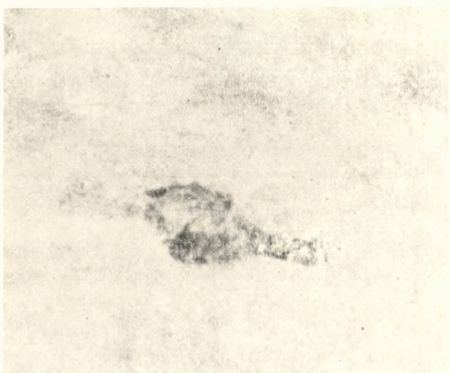

Fig. 4. N. I. Calcium deposits in the granules of conjunctival mastocytes. Kossa's method. ×1000

Discussion

It is common knowledge that renal failure may be associated with hypocalcaemia and hyperphosphataemia, resulting in secondary or renal hyperparathyreoidism. On the other hand, the compensatory parathyroid hyperfunction causes hypercalcaemia, in which case calcium depositions involving the conjunctiva represent in fact metastatic calcifications. The present Case 1 belonged to this type. The process may be interpreted by SELYE's calciphylaxis. This phenomenon has been described in humans by REES and COLES [11] who noted calcium deposits in uraemic patients at the injection sites of iron-dextran.

However, deposition of calcium in the presence of a low or normal serum calcium level still lacks adequate explanation. The pertaining literature gives no satisfactory answer to the question. Some authors including HEATH [8], ABRAMS [1], EASTERBROOK and MORTIMER [6] also fail to account for the pathomechanism of conjunctival calcification. On the other hand, BERLYNE and SHAW [2] regard the high Ca × P product as an essential factor. All their patients with conjunctival calcium deposits, even those with hypocalcaemia, had a high Ca × P product, and they regard the high plasma inorganic phosphate level as responsible for this elevation. Case 4 in the present series corresponded to this type. On the other hand, in Cases 2 and 3, a normal serum phosphate level was found and the hypocalcaemia.

DHERMY [5] reported a case of palpebral calcification of the Winer-type with a normal blood calcium level. He emphasized that the degeneration of collagen is accompanied by a release of calcium from the tissues; its precipitation may be promoted by local pH changes.

Considering all the above, it clearly emerges that the red eyes, as concerns their pathomechanism, fall into different types. What they have in common is some unidentified factor which induces the deposition of calcium in the conjunctiva. This factor may be sought in a physical irritation of some kind, for instance excessive cooling, evaporation, dust, smoke, etc., acting as a microtrauma to the exposed conjunctival area.

The observation that the conjunctival mastocytes also revealed calcium deposits invites further investigations into the part played by these cells and the possible involvement of mastocalciphylaxia in the phenomenon of red eyes.

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THE HAEMODYNAMIC BASIS OF ANGINAL RELIEF PRODUCED BY STIMULATION OF THE CAROTID SINUS NERVE

(EFFECT OF CAROTID SINUS STIMULATION ON CIRCULATION AND ON THE DISTRIBUTION OF CARDIAC OUTPUT)

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The effects of stimulation of the carotid sinus nerve on the blood flow of the essential organs (heart, brain, kidney, muscles, skin) and on haemodynamic regulations have been examined in 16 dogs, with 40 other dogs serving as controls.

The carotid sinus was stimulated by a Medtronic radiofrequency carotid sinus stimulator. The parameters were the following: 3 to 6 mV voltage, 30 to 50 Hz frequency, 350 μsec. impulse duration. Cardiac output and blood flow were determined by the dye-dilution method with Evans-blue, organ blood flow by ^{86}Rb and ^{131}I-antipyrine. A significant fall in cardiac output, peripheral vascular resistance and velocity of blood flow was demonstrable during stimulation, with the mean reduction in pressure work calculated for the left ventricle amounting to 40%. Despite the reduction in blood pressure and cardiac output there was an increase in coronary flow during stimulation, in contrast to cerebral, renal, cutaneous and muscular flow which exhibited a slight fall. The cardiac fraction of cardiac output showed a marked increase in response to carotid sinus stimulation.

The observation that stimulation of the carotid sinus nerve is apt to relieve anginal pain is not a new one [1]. In recent years electric stimulation of the carotid sinus has been successfully employed in drug-resistant cases of angina pectoris [2, 3, 4, 5, 6]. The present authors undertook implantation of a carotid sinus electric stimulator (Medtronic radiofrequency carotid sinus stimulator) with complete success in a drug-resistant case of angina pectoris. Despite extensive research work on the subject, the haemodynamic factors accounting for the antianginal response to stimulation of the carotid sinus nerve are not fully understood. The aim of the present study was to gain information on the changes of the essential areas of blood supply and of the distribution of cardiac output to the individual organs consequent upon stimulation of the carotid sinus nerve.

Material and methods

Fifty-six mongrel dogs of either sex were studied. In 16 animals the haemodynamic studies were performed during carotid sinus-nerve stimulation, 40 served as controls. Cardiac output, circulation time and the volume of circulating blood were determined by the dye-dilution method, with Evans-blue (2 mg per kg body weight intravenously). Blood flow of heart, brain, kidney, muscles and skin were measured by SAPIRSTEIN's indicator of frac-

tional distribution method [8], by the joint use of ^{86}Rb and ^{131}I-antipyrine [9]. The organ fractions of cardiac output were calculated from the values of cardiac output and of regional blood flow per 100 g organ weight. For stimulation, the carotid sinus nerve was exposed in the carotid bifurcation and the electrode was placed round it. The manipulations were performed under superficial pentobarbitural anaesthesia (20 mg per kg body weight intravenously). The stimuli were applied with a carotid sinus stimulator, voltage being 3 to 5 V, the frequency of stimuli 30 to 50 per second, duration of impulses 350 microseconds. Stimulation was followed by a fall in blood pressure and slowing of the pulse rate (checked by ECG) averaging 20 to 30 and 15 to 20%, respectively.

For the analysis of the results, Student's two-sample *t*-test was used.

For reference purposes, the data of human haemodynamic studies performed prior to and during stimulation of the carotid sinus nerve are also given. Cardiac output, circulating blood volume and circulation time were measured with the Evans-blue technique (50 mg δ intravenously).

Results

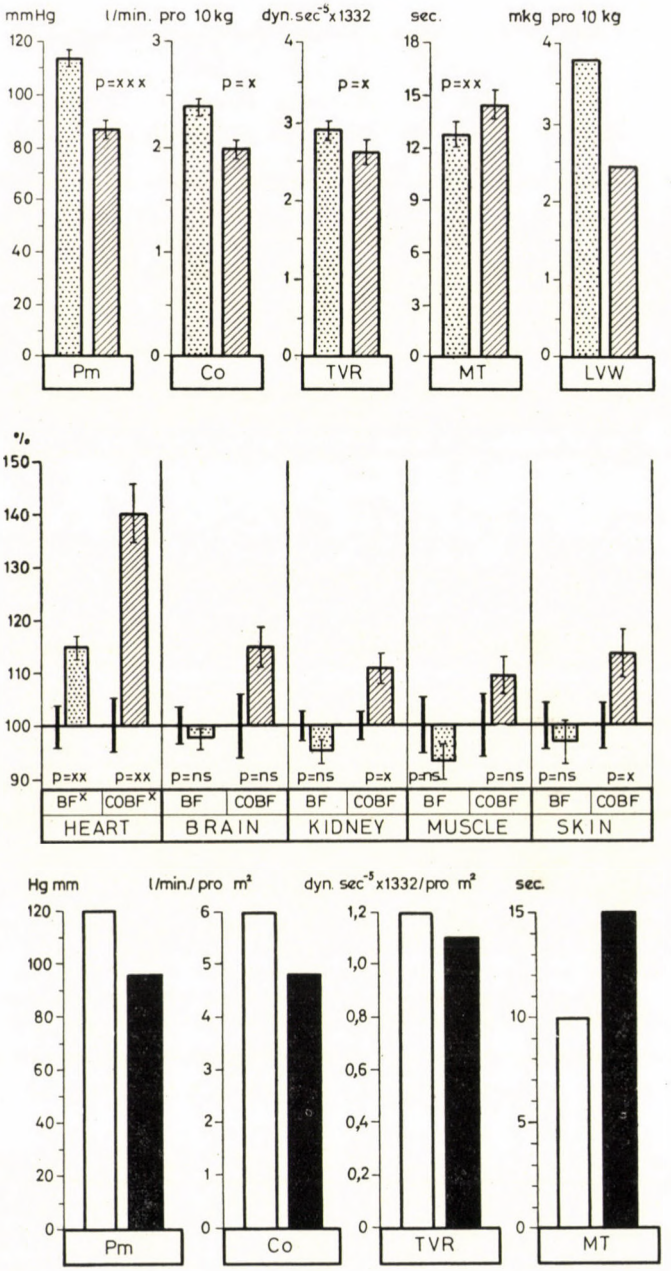
Results of the animal experiments are shown in Figs 1 and 2.

Fig. 1 shows the changes produced in systemic circulation. As a result of stimulation of the carotid sinus nerve (cross-hatched columns) blood pressure, cardiac output and peripheral arterial resistance significantly diminished, as opposed to a prolongation of circulation time. All these changes were significant statistically.

Left ventricular pressure work was calculated from mean arterial pressure and cardiac output (in view of the normal haemodynamic state of the animals, the left ventricular end-diastolic pressure was considered zero). As it can be seen, stimulation of the carotid sinus nerve relieved the pressure work imposed on the heart by nearly 40% (Fig. 1).

Fig. 2 shows the individual organ flows and the organ fractions of cardiac output in response to stimulation of the carotid sinus, expressed in per cent of the corresponding parameters of the control group, the mean values of this group being taken as 100% (Fig. 2). From the graph it emerges that coronary blood flow increased by 15% in response to carotid sinus stimulation despite a fall in blood pressure and in cardiac output, whereas brain, kidney, muscle and skin flow slightly diminished. The increase in cardiac blood flow was significant statistically. The cardiac fraction of cardiac output also showed a 40%, statistically significant, increase. A slight increase was demonstrable in the cerebral, renal, muscular and cutaneous fractions of cardiac output, with the exception of the renal fraction, the value for which failed to attain the level of significance.

Fig. 3 represents the response of the haemodynamic parameters of a patient with grave angina pectoris to successful electric stimulation of the carotid sinus nerve (Fig. 3). It can be seen (full columns) that, similarly as in the laboratory animals, stimulation of the carotid sinus nerve produced a substantial fall in blood pressure, peripheral vascular resistance and velocity of blood flow.



Abbreviations for figures 1—3.

BF	=	organ blood flow
COBF	=	organ fraction of cardiac output
Pm	=	mean arterial blood pressure
CO	=	cardiac output
TVR	=	total vascular resistance
MT	=	mean transit time
LVW	=	calculated work of left ventricle

Discussion

On the evidence of the present study, carotid sinus-nerve stimulation results in changes in the haemodynamics of systemic circulation to the profit of blood flow of the essential organs of circulation and also produces slight modifications in the haemodynamic regulations. A fall in arterial mean pressure and in cardiac output has been noted, in accordance with the present results, by other workers as well. BEVEGARD et al. [10], GRIFFITH and SCHWARTZ [11], REICH et al. [12], EPSTEIN et al. [13], ROTHFELD et al. [14], LOWN and LEVINE [15] as well as GERSMEYER [16] found a decrease in pulmonary pressure in response to stimulation of the carotid sinus nerve.

The findings in respect of the effect of carotid sinus-nerve stimulation on coronary flow are less consistent. While, according to FALICOV et al. [17], coronary blood flow diminishes, according to VATNER et al. [18, 19] it remains generally unaffected, despite a decrease in blood pressure, though increasing in a number of cases. DE GEEST et al. [20] noted a fall in coronary resistance response to stimulation of the carotid sinus nerve [20].

The haemodynamic responses of the other organs are less amply documented in the literature. According to ERNSTING and PARRY [21], ZINGHER and GRODINS [22], GAULT et al. [23] and to BEVEGARD and SHEPHERD [10], peripheral (extremital) vascular resistance significantly diminishes during stimulation of the carotid sinus nerve. A fall in renal blood flow parallel with stimulation of the carotid sinus has been observed by TUCKMAN et al. [24] and NEISTADT and SCHWARTZ [25]. RESNICOFF et al. [26] noted a regional increase in peripheral vascular resistance within the area of blood supply of the vertebral artery, in opposition to the area of carotid blood supply where the peripheral vascular resistance was unaffected.

The present findings seem to indicate that stimulation of the carotid sinus nerve not only produces changes in blood circulation but also modifies the distribution of cardiac output in the individual organs, particularly as concerns the increase in the cardiac fraction during stimulation of the carotid sinus. Coronary flow, i.e., the nutritive blood supply to the heart, greatly increases despite a substantial simultaneous fall in blood pressure and cardiac output.

The haemodynamic changes demonstrated by the present study thus account for the relief of anginal pain produced by stimulation of the carotid sinus nerve. The work imposed on the left ventricle greatly diminishes as a result of the fall in cardiac output, in arterial pressure and in peripheral vascular resistance. Moreover, despite the fall in blood pressure and in cardiac output, coronary blood flow increases in consequence of a substantial dilatation of the coronary vascular bed. A modification of these two factors by the carotid sinus stimulation thus benefits the oxygenation of the heart muscle.

*

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ECG AND VCG IN ACCIDENTAL AND EXPERIMENTAL HYPOTHERMIA

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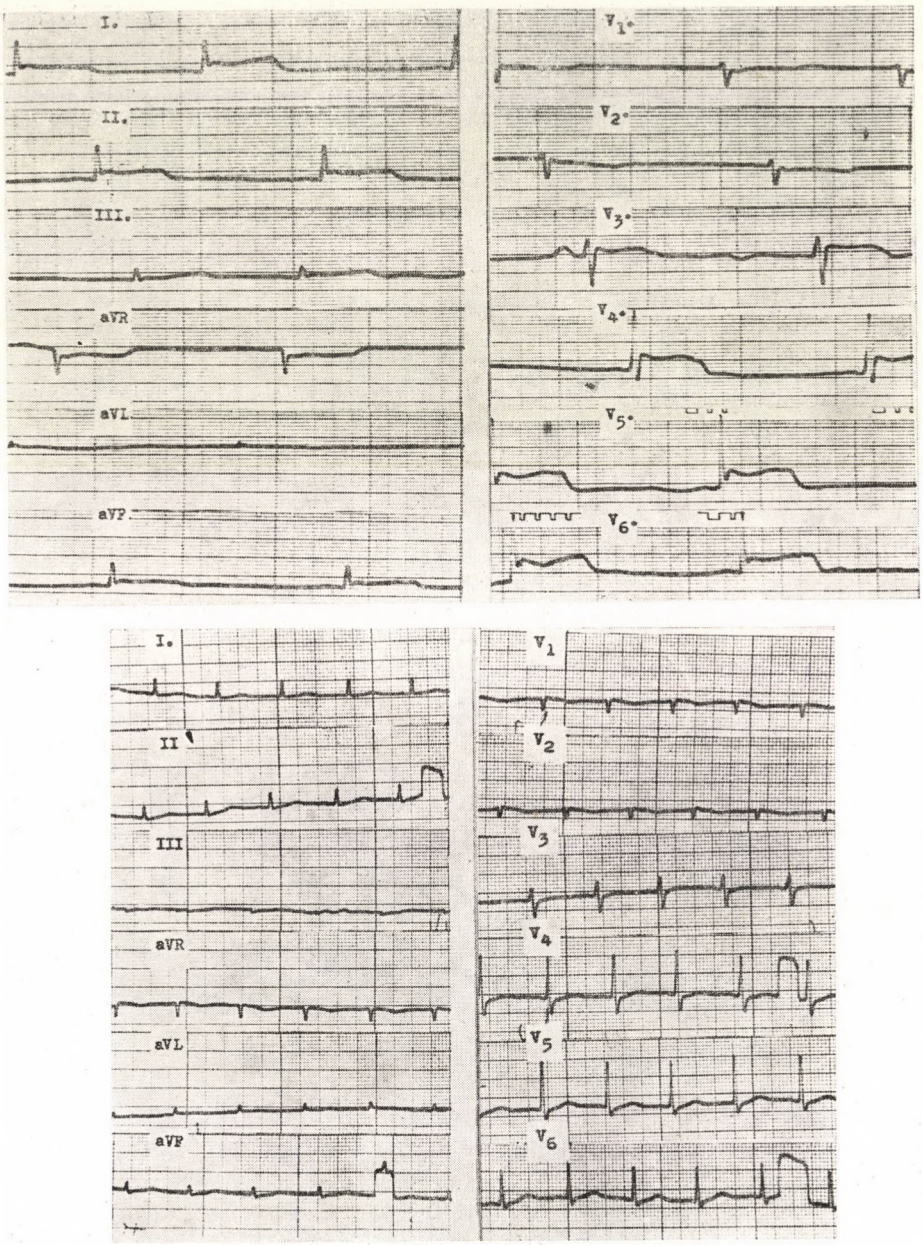
The ECG features of accidental hypothermia are presented in the light of five observations, consideration being given to possible relationships between hypothermic ECG abnormalities and ventricular fibrillation. The ECG and VCG abnormalities associated with hypothermia have been reproduced in dog experiments and the exact location in time and space of the J deflection has been attempted by epicardial leads and its derivatives. It has been found that the J deflection belongs partly to depolarization, partly to repolarization and that it is due to an extreme delay in the activation of the posterobasal region.

Hypothermia, whether accidental or artificial, is associated with various electrophysiological changes. Ventricular fibrillation is the gravest hazard of surgical hypothermia [4, 8, 19, 28, 33]; it constitutes the direct cause of death in accidental hypothermia as well as in hypothermia resulting from grave hypothyroidism [10, 11, 13, 24, 32].

TOMASZEWSZKY [39] was the first to describe an ECG change in a case of accidental hypothermia (1939), in particular a characteristic slow deflection between the QRS complex and the ST segment. This could then be reproduced by other workers in various animal species under experimental conditions [8, 11, 12, 30, 23, 29, 40]. The abnormal ECG features of hypothermia, including a widening of QRS, abnormal Q, prolonged PQ-, QT- and RR-intervals, particularly an elevation of the J junction giving origin to the deflection wave ST (deflection J) have been among the most widely debated issues of experimental hypothermia in the last fifteen years. OSBORN [29] regards the relationship between the appearance of the J deflection and of ventricular fibrillation as established and ascribes these abnormalities to hypoventilation associated with hypothermia and to consecutive acidosis. While WYNNE, FULLER and SZÉKELY [47] as well as SCHWAB and his associates [35] also relate the J deflection to ventricular fibrillation, other workers [10, 33, 40] have questioned this relationship. EMSLIE-SMITH, examining the vector-cardiogram in one case of hypothermia, demonstrated an extra loop, the J loop, corresponding to the J deflection, between the QRS and T loops [12].

We had five patients with accidental hypothermia at our Intensive Care Unit in the period 1970/71. Rectal temperature on admission was between



Figs 1, 2. G. R., female, 80 years. ECG tracings in hypothermia (24°C) and after successful rewarming (35.7°C). J-deflection characteristic of hypothermia is most obvious in V_{4-5}. ECG obtained after rewarming no longer shows these abnormalities

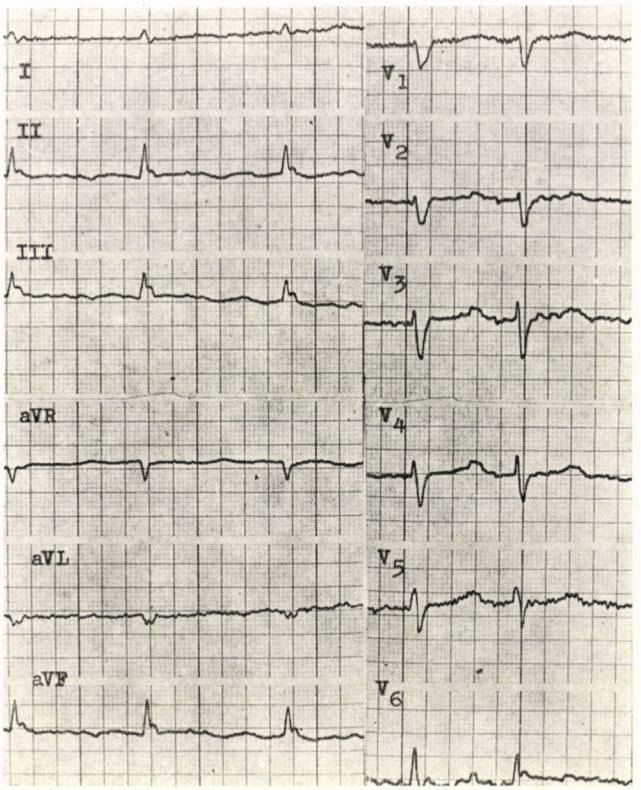


Fig. 3. G. F., male, 66 years. Hypothermic ECG with a distinct J deflection in II, III and aVF. The saw-toothed appearance of the baseline is due to muscle tremor

24° and 31°C. Repeated 12-lead ECG-tracings were recorded under monitoring the rectal temperature and the ECG. This was completed in one case by the orthogonal Frank leads and by VCG. The ECG findings were evaluated in the context of the clinical picture and of laboratory evidence.

The ECG findings included bradycardia and a lengthening of QT related to the degree of hypothermia, in two cases auricular fibrillation, in three cases prolonged QT, in two cases appearance of an abnormal Q, and in all of the cases an elevation of the J junction and the appearance of a J deflection (ST deflection). This J deflection was most obvious in the central praecordial leads.

Figs 1 and 2 show the ECG tracings of G. R., a 80-year-old female patient with barbiturate poisoning. The first tracing was made at admission in hypothermia (rectal temperature 24°C) and the other in normothermia. The first ECG made at a paper speed of 25 mm/sec revealed auricular fibrillation with extreme bradycardia (18 per min). QRS measured 0.12 sec; QT,

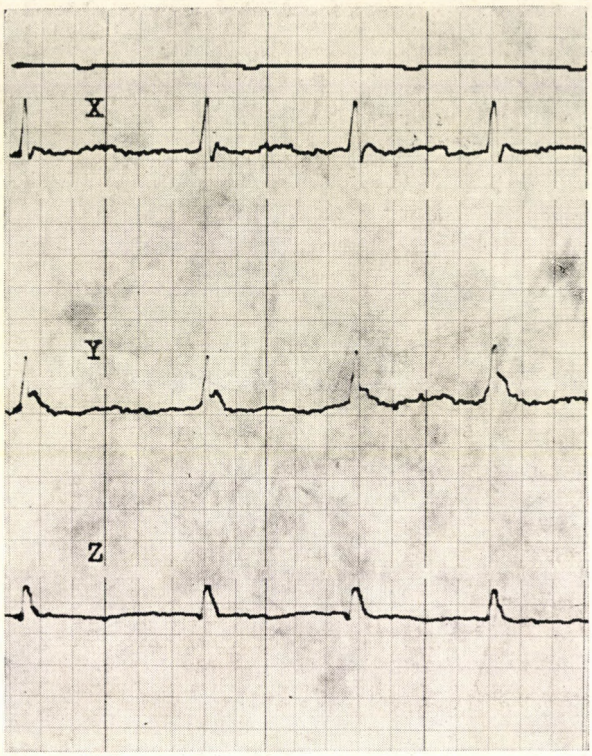


Fig. 4. Same patient as in Fig. 3. Orthogonal leads X, Y and Z in hypothermia. Elevation of junction J and the J deflection are most obvious in lead Y

0.92 sec. In leads I, II, III, aVF and V_{3-6} there was ledging of the base of QRS, forming the point of origin of an extra J deflection. After rewarming to 35.7°C the ECG showed sinus rhythm, a heart rate of 88. PQ measured 0.14 sec; QRS, 0.06 sec; QT, 0.38 sec.

The ECG in Fig. 3 belongs to the 66-year-old male patient W. F., who had a rectal temperature of 28.5°C on admission. ECG revealed auricular fibrillation with bradycardia of 60 per min and a vertical position of R. In leads II, III and aVF the descending limb of QRS was ledged and formed the point of origin of a J deflection. The fine oscillations on the baseline were caused by muscle tremor [24, 32] (Fig. 4). The Frank orthogonal leads shown in Fig. 4 lead X and Y clearly show an elevation of the J junction (Fig. 5). The VCG seen in Fig. 5 revealed a hemicircular concavity of the terminal section of QRS (J loop) in all three planes, particularly in the horizontal and the sagittal plane. The J loop appeared before the completion of ventricular depolarization, as a sign of which the descending limb of R failed to return to its origin in the orthogonal Y lead.

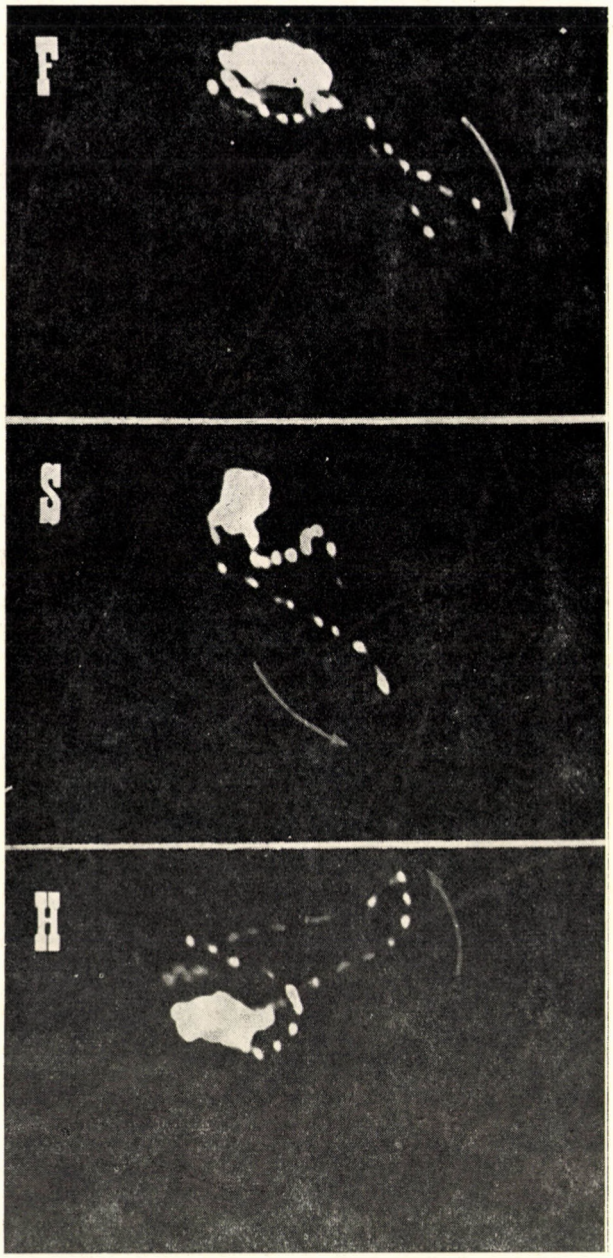


Fig. 5. **Vector-cardiogram in the frontal sagittal and horizontal planes of the same patient in hypothermia (28.5 C). The terminal portion of QRS displays a hemicircular concavity (J loop) in all three, particularly in the sagittal and horizontal, planes**

Experimental studies

It was sought to study the ECG abnormalities found in hypothermia more closely under experimental conditions, in particular, to define the exact time and space relationships of the J deflection which earlier workers have failed to clarify [10, 14, 29, 32, 46].

Six appearently healthy mongrel dogs of either sex weighing between 10 and 14 kg were studied. A cocktail made up of 100 mg pethidin, 50 mg promethazine and 25 chlorpromazine was administered, the femoral arteries and veins were prepared and with the rectal temperature still ranging between 37.5 and 38.0°C, a corrected orthogonal ECG according to McFee and Parungao [25] and the VCG, were recorded, then after succinyl-relaxation controlled respiration was applied with a 2 : 1 mixture of N_2O and O_2. Left thoracotomy was performed while the animals were still normothermic, the heart was exposed, and epicardial unipolar and bipolar electrodes were fixed to determined points of the left ventricle. The recordings were made with a 3-channel direct-writing Hellige apparatus, at a paper speed of 100 mm/sec. For the bipolar electrodes we used a plantinum wire 0.5 mm in diameter with rounded tips mounted in a plastic frame. These were placed 1 mm apart and connected to standard lead II. For the unipolar leads we used a stainless steel electrode 1 mm in diameter, which was connected to the chest electrode. Passive derivatives by means of an RC circuit with a time-constant of 1 msec were recorded synchronously with the epicardial tracings. After closure of the thoracotomy wound, local cooling was performed by immersion in ice cold water. Respiratory rate was reduced in proportion to the fall of rectal temperature. At 29°C the animals were removed from the cooling bath and this was followed by a spontaneous further fall to 28°C. This temperature was maintained if necessary with the aid of warmed perfusions. The control recordings were derived from identical points, partly with open, partly with closed chest. The tracings were analysed on the basis of their configuration and quantitative character. The studies were completed by acid– base-, blood-gas and electrolyte measurements.

The ECG-abnormalities revealed by these experiments were consistent with those found in human accidental hypothermia.

Fig. 6 shows leads X, Y and Z in normothermia and hypothermia. On comparison of the two tracings we find a conspicuous increase in amplitude of P, particularly in lead Y, and the appearance of a deep Q in lead X. QRS increased from 0.09 sec to 0.12 sec and QT from 0.22 sec to 0.34 sec. In all three leads there is an elevation of junction J, and the appearance of a J-deflection of left, posterior and downward orientation (Fig. 7). The VCG in the

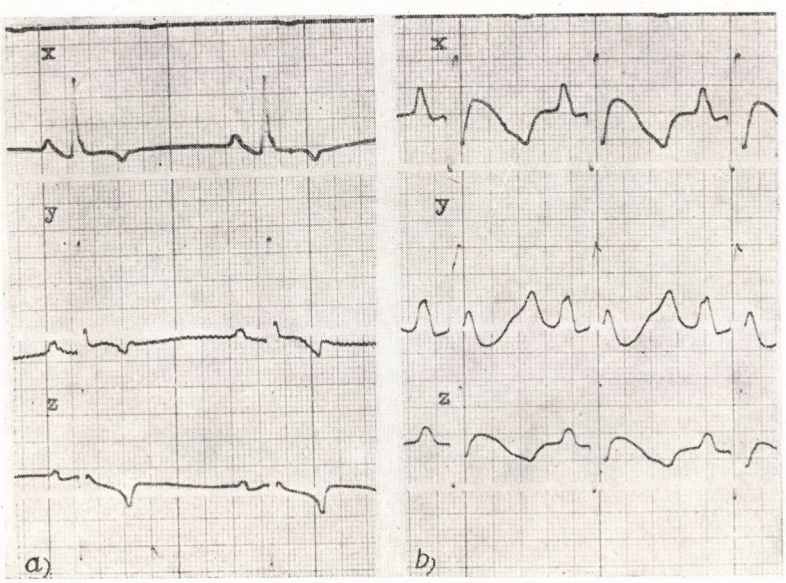


Fig. 6. Comparison of leads X, Y and Z in normothermia and hypothermia (28.0°C). Widened QRS, prolonged Qt. Deflection J oriented to the left, posteriorly and downward can be seen in all three leads: *a)* normothermia (37°C), *b)* hypothermia (28°C)

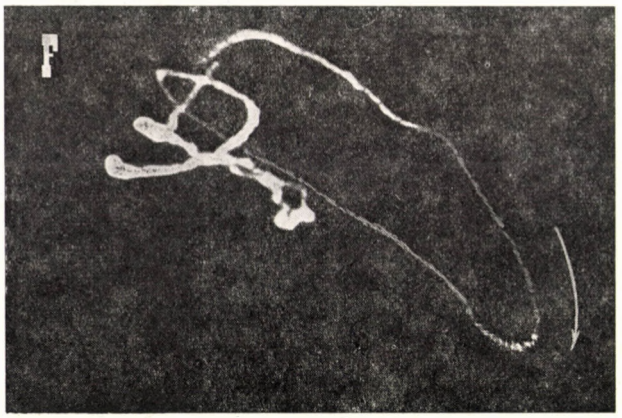


Fig. 7. VCG in the frontal plane in experimental hypothermia. The loop of clockwise inscription gives origin at its terminal portion to an extra loop (J loop), and loop T starts only afterwards

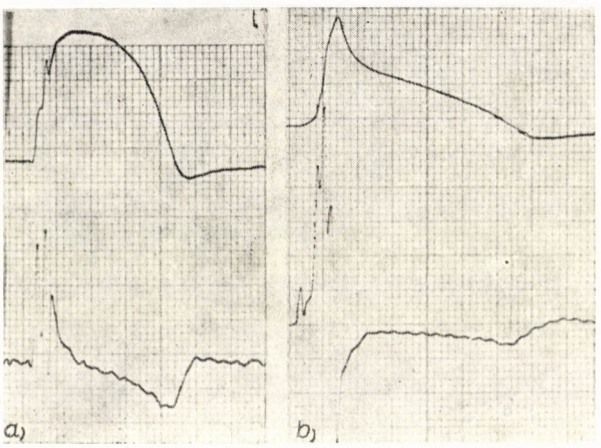


Fig. 8. Bipolar epicardial leads and their first derivatives in normothermia and hypothermia. After cooling, the steepness of the ascending limb diminishes while the action potential increases in duration: *a)* normothermia (37°C): *b)* hypothermia (28°C)

frontal plane seen in Fig. 7 distinctly shows an extra, U-shaped loop (J loop) arising from the terminal part of the loop of clockwise inscription, while the T loop starts only afterwards.

The epicardial leads derived from the surface of the left ventricle and their first derivatives in time give closer details of the spread of impulse (Fig. 8). In Fig. 8 the bipolar leads have been compared in normothermia and in hypothermia. It is seen that after cooling the ascending limb becomes less steep, the duration of the action potential being prolonged. In the unipolar leads cooling is followed by the disappearance of the physiologic T deflection and by the appearance of a wide, upright deflection J (Fig. 9). The tracing provides valuable clues to the analysis of the complex. The second upright peak points to the elevated J junction, thus marking out the turning point between depolarization and repolarization. There is a delay in depolarization between the free wall of the left ventricle and its posterobasal region. This gives the best projection in the unipolar epicardial leads where the intrinsicoid deflec-

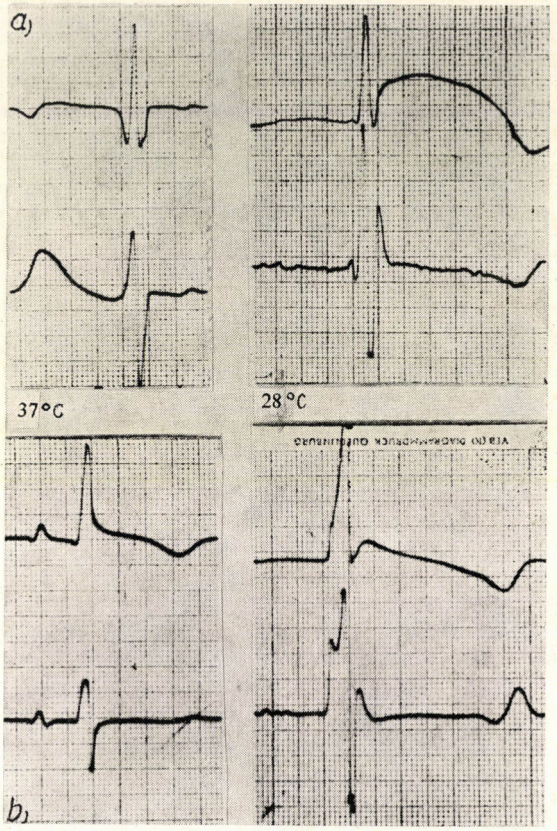


Fig. 9. Unipolar epicardial leads and their first derivatives in normothermia and hypothermia. The second upward peak of the derived lead points to the elevated J junction, thus clearly marking the turning point between depolarization and repolarization. Depolarization is delayed between the free wall and the posterobasal region of the left ventricle, accordingly the intrinsicoid deflection is also delayed: *a)* free wall of left ventricle, *b)* posterobasal region

Table I

Unipolar

Case	Electrodes	Basic values			28°C		
		R/mV	QT (sec)	dV/dt mV/sec	R/mV	QT (sec)	dV/dt mV/sec
1	Left ventricle, anterior	19	0.27	1200	19	0.40	700
	Posterobasal	19	0.27	1000	26	0.40	600
2	Left ventricle, anterior	9	0.25	1400	10	0.35	800
3	Left ventricle, anterior	21	0.20	1400	20	0.30	1100
4	Left ventricle, anterior	15	0.30	900	17	0.38	800
5	Left ventricle, anterior	10	0.32	1000	11	0.40	900
6	Left ventricle, anterior	16	0.28	800	15	0.35	700

Table II

Bipolar

Case	Electrodes	Basic values			28°C		
		R/mV	Duration (sec)	dV/dt mV/sec	R/mV	Duration (sec)	dV/dt mV/sec
1	Left ventricle, anterior	19	0.21	700	15	0.38	250
	Posterobasal	29	0.21	600	—	—	—
2	Left ventricle, anterior	25	0.24	1300	20	0.39	800
3	Left ventricle, anterior	28	0.27	700	25	0.40	500
4	Left ventricle, anterior	22	0.25	1200	25	0.38	700
5	Left ventricle, anterior	16	0.27	900	19	0.35	600
6	Left ventricle, anterior	22	0.27	1150	19	0.36	800

Quantitative analysis of the unipolar and bipolar epicardial leads shows that the duration of QT, more correctly of the action potential, increases in all of the cases. Correspondingly, the fraction dV/dt expressed in mV/sec diminishes.

tion shows a delay in the posterobasal region. In accordance with this, the derivative give a smaller dV/dt value.

Quantitative evaluation of the epicardial leads has been set out in close detail in Table I and II. QT, the duration of action potential is significantly prolonged under hypothermia all throughout and owing to a delay in depolarisation the dV/dt value expressed in mV/sec diminishes.

Discussion

Hungarian literature dealing with the cardiovascular aspects of hypothermia [5, 15, 17, 21, 26, 37, 38, 43, 44] offers no adequate information on the ECG abnormalities associated with the condition. On the evidence of the observations by Büky [6], intravenous administration of magnesium reduces the hazard of ventricular fibrillation, hypomagnesaemia being theoretically apt to produce intracellular potassium loss [2, 30, 45]. While Berne [3] attributes the low fibrillation threshold to an impaired ATP activity of the heart, Torres, Angelakos, Székely and Wynne [40 1, 36,] assume, on the contrary, the presence of intracellular hypopotassaemia. As it has been pointed out by Emslie-Smith [10], deflection J is invariably present below 31.5°C, moreover, its amplitude is related to the degree of hypothermia, it may exceed the QRS complex in magnitude and it may be associated with an inverted T. The ECG changes are reversible, yielding gradually to successful rewarming. They lack any characteristic light-microscopic substrate [20, 31]. Various explanations, such as conduction disturbances [32, 39], subepicardial ischaemia [29, 47], disorders of acid–base [42] or electrolyte [2, 30, 36] balance or of enzymatic activity [7, 9, 34, 48] have been put forward to account for the changes

but all are contestable on theoretical grounds. REE [32] concluded from the analysis of a single hypothermic ECG tracing that deflection J originated in an extreme delay in depolarization of the posterobasal region. The results of our present studies are consistent with this interpretation. The unipolar tracing and its derivatives clearly bring out the coincidence in time of the appearance of deflection J with depolarization of the posterobasal region which shows a substantial delay.

It has been confirmed by the present results that deflection J coincides partly with depolarization, partly with repolarization. These two phases are separated by the J junction which, though not apparent in the scalar leads, is clearly displayed on the VCG in the form of a slight angular distortion of the J loop, and can be located with the aid of the ECG, more precisely, of the first derivatives of the epicardial leads.

Clarification of the relationship between ECG changes and ventricular fibrillation associated with hypothermia requires further studies.

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URINARY OESTROGENS IN NORMAL EGYPTIAN SUBJECTS AND IN PATIENTS WITH BILHARZIAL HEPATOSPLENOMEGALY

By

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Total oestrogen, oestrone, oestradiol and oestriol fractions were estimated in normal Egyptians and patients with hepatosplenomegaly. The patients were divided into 3 groups; adult males, adult females and postmenopausal women. There was a tendency to high oestrogen levels in males and postmenopausal women. The increase was significant in the oestradiol fraction. In adult females, total oestrogen and the oestriol fraction were significantly lower.

No correlation could be established between the feminine clinical features and the oestrogen level, the result of liver biopsy and that of liver function tests.

The role of the liver in oestrogen metabolism is well-known. Bilharziasis, a disease endemic in Egypt, affects the liver and this may result in a failure of proper oestrogen inactivation and the occurrence of manifestations of hyperoestrogenism such as gynaecomastia, testicular atrophy, hypotrichosis, spider angiomas and palmar erythema [4, 7].

Several workers have reported elevated levels of oestrogenic metabolites in the urine of cirrhotic patients [1, 9, 11]. In contrast, others found a normal blood and urinary oestrogen level [2]. Hence, the question whether the altered oestrogen metabolism accounted for some clinical symptoms of liver disease is a controversial one [4, 7].

With the introduction of new and delicate methods of oestrogen assay, the question has been studied anew, and it arose that the answer may not depend on the total value but on the relative values of the different oestrogenic constituents of variable activity and different rates of degradation and disposal.

The aim of the present work was to study the urinary oestrogens, i.e., oestriol, oestradiol and oestrone, in normal subjects and patients with liver disease and to try to correlate the findings with the clinical, biochemical and pathological symptoms.

Material and methods

Two groups of human subjects were examined. A total of 57 normal healthy subjects, consuming normal diets, without any endocrine disease were classified into three groups. The first group consisted of 15 normal male volunteers aged 21—33 years, in army service.

The second group was made up of 22 females 19—36 years of age with a history of normal regular menses since menarche. From this group 24-hour-urine samples were collected on the 14th day of the cycle.

The third group comprised 20 menopausal and post-menopausal female subjects 35—62 years of age. From these, 24-hr-urine samples were collected without adding a preservative and kept in the deep freeze until processed.

Cases were selected from inpatients suffering from chronic liver disease with hepatosplenomegaly. They were not affected by any other metabolic or endocrine disease. These subjects were classified into 3 groups.

The first group consisted of 10 male patients aged 20 to 35 years with bilharzial hepatosplenomegaly. Many of them were in an advanced stage of the disease. They were coming from endemic areas and had a history of bilharzial infection and treatment. Liver biopsy was done (4) to show the nature of the lesion. History and clinical data were recorded, with emphasis on the symptoms and signs related to oestrogens.

The second group was made up of 10 female patients with bilharzial hepatosplenomegaly, aged 19 to 36 years. At this age, women are usually less exposed to infection than males. In these patients, no gynaecological or endocrine manifestations were observed. 24 hr urine was collected on the 14th day of the menstrual cycle in view of the oestrogen peak at ovulation.

The third group consisted of 9 menopausal and post-menopausal patients, aged 54—62 years, with liver cirrhosis. Most of them were advanced cases of non-bilharzial cirrhosis as evidenced by history and liver biopsy. 24-hr urine was collected without adding a preservative and kept in the deep freeze until processed.

The following liver-function tests were carried out in every patient: serum bilirubin, total plasma protein, albumin—globulin ratio, thymol and zinc-sulphate turbidity tests, alkaline phosphatase, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase and prothrombin time.

The history and clinical data were recorded and analysed.

The three classical oestrogens were determined in the 24-hr-urine samples by a modified procedure based on the method of MILLER et al. [6].

The method consisted of acid hydrolysis of 100 ml urine after the addition of 0.2 ml formalin, extraction with dichloromethane, and preliminary purification by solvent partition. Then the residue was subjected to paper partition chromatography. After elution, the oestrogens were further freed from contaminations by silica-gel-column chromatography instead of the thin-layer chromatography employed by MILLER [6]. Recovery of the classical oestrogens was more favourable by column than thin-layer chromatography. The column was made by mixing of one g silica gel (100—200 mesh) to benzene, and transferred to a disposable pipette.

Different eluting solvents were tried using dichloromethane and methanol in varying proportions. The optimum total volume of eluent was also studied. The following eluents ensured the maximum recovery of added oestrogen.

To confirm the nature of the oestrogen recovered from the column, the eluted sample was evaporated to a residue that was run against authentic oestrogen standards on thin-layer plates in two different solvent systems. Each sample had the same Rf value as the corresponding authentic oestrogen standard.

Oestrogen	Eluent	Recovery per cent
Oestrone	30 ml of 1% methanol-dichloromethane	71
Oestradiol	30 ml of 4% ,, ,,	76
Oestriol	30 ml of 10% ,, ,,	84

Results and comment

Results are recorded in Table I—VI with their statistical analysis, while Figures 1, 2 and 3 compare the urinary oestrone, oestradiol, oestriol and total oestrogens in the normal subjects and the patients with liver disease. There was a slight increase in total oestrogens in the male patients with hepatosplenomegaly (16, 54) as compared with the level in the normal subjects (13, 9); the increase was not significant statistically ($P > 0.1$), yet the figures for oestradiol showed a statistically significant increase ($P < 0.01$).

Table I

Urinary oestrogens in a group of normal Egyptian males

Case No.	Age	Oestrone	Oestradiol	Oestriol	Total oestrogens
		E_1 μg/24 hr	E_2 μg/24 hr	E_3 μg/24 hr	μg/24 hr
1	26	7.38	2.02	7.25	16.65
2	29	5.91	1.98	7.90	15.79
3	22	2.03	3.10	2.30	7.34
4	24	7.82	2.30	3.70	13.82
5	32	3.20	3.80	1.70	8.70
6	27	3.20	4.00	10.60	16.80
7	22	8.10	1.90	8.20	18.20
8	26	0.96	3.20	4.31	8.47
9	33	5.71	4.52	4.61	14.84
10	21	4.40	5.05	7.67	17.12
11	25	5.96	3.39	9.35	18.70
12	23	9.10	2.40	3.42	14.92
13	24	1.78	2.84	4.34	8.96
14	32	6.04	5.78	3.44	15.26
15	28	3.64	4.23	4.97	12.84
Mean		4.95	3.37	5.58	13.90
S.D. ±		2.57	1.18	2.64	3.72
S.E. ±		0.66	0.31	0.68	0.96
% of total		35.7	24.2	40.1	

In the same group, oestrone and oestriol fractions were not significantly affected ($P > 0.5$, > 0.10 respectively).

Among the patients studied (Table IV), 4 cases (2, 4, 9, 10) showed the highest urinary oestrogen levels. Three of these cases (4, 9, 10) showed endocrine manifestations: in Case 4 there was a loss of axillary and pubic hair

Table II

Urinary oestrogens in a group of normal young Egyptian females (urine collected on 14th day after the beginning of menstruation)

Case No.	Age	Oestrone	Oestradiol	Oestriol	Total oestrogens
		E_1 μg/24 hr	E_2 μg/24 hr	E_3 μg/24 hr	μg/24 hr
1	28	14.62	12.62	42.03	69.27
2	26	8.34	15.73	35.94	60.01
3	32	10.41	21.46	57.41	89.23
4	19	13.06	8.71	32.42	54.19
5	25	12.14	13.34	37.67	63.15
6	28	7.65	16.61	28.34	52.60
7	35	6.70	26.00	79.2	111.90
8	26	7.40	21.00	38.20	66.60
9	29	8.70	15.80	20.30	44.80
10	27	10.10	13.10	15.50	38.70
11	27	5.60	10.60	41.50	57.70
12	28	9.67	3.92	70.43	84.20
13	23	12.55	8.41	53.68	74.64
14	33	18.50	13.40	34.10	66.90
15	24	12.90	12.70	18.70	44.30
16	30	7.90	18.60	43.30	69.80
17	27	9.32	10.23	32.91	52.46
18	36	15.16	6.76	345.32	67.24
19	21	6.34	9.24	33.96	49.54
20	26	11.78	15.06	18.63	45.47
21	25	6.42	17.39	50.36	74.17
22	26	8.07	14.97	41.04	94.08
Mean		10.11	14.38	42.32	66.81
S.D. ±		3.33	8.59	17.29	18.17
S.E. ±		0.71	1.83	3.68	3.87
% of total		15.1	21.5	63.4	

with impotence; in Case 9, gynaecomastia, Case 2 displayed no change, although the total oestrogens were high.

In those cases which displayed endocrine symptoms, the oestradiol level was high and liver function tests were moderately positive. Cases 1, 5, 6 and 7 showed clinically advanced liver disease with ascites; the last three had jaundice too, and Case 7 was in hepatic coma. Only Case 6 showed palmar erythema and spider naevi. Liver-function tests were pathological in these

Table III

Urinary oestrogens in a group of post-menopausal women

Case No.	Age	Oestrone	Oestradiol	Oestriol	Total oestrogens
		E_1 μg/24 hr	E_2 μg/24 hr	E_3 μg/24 hr	μg/24 hr
1	58	3.25	1.65	5.62	10.52
2	60	1.64	2.42	6.38	10.44
3	57	4.37	1.76	3.18	9.31
4	53	3.30	2.70	3.80	9.80
5	58	2.50	3.80	3.40	9.70
6	57	4.96	2.10	5.70	12.76
7	54	5.10	4.90	6.72	16.78
8	59	2.41	1.41	7.07	10.89
9	60	4.03	0.83	2.37	8.13
10	56	7.19	4.43	1.11	12.73
11	58	1.03	2.26	4.73	8.02
12	62	2.10	1.25	7.35	10.70
13	58	3.36	2.75	5.76	11.87
14	55	5.06	3.41	1.61	10.08
15	54	4.35	3.27	2.46	10.08
16	58	5.26	3.44	4.41	13.11
17	57	0.69	4.21	5.67	10.57
18	60	6.07	1.39	6.34	13.80
19	59	2.96	3.86	7.62	14.44
20	58	1.72	2.94	3.28	7.96
Mean		3.56	2.81	4.77	11.14
S.D. ±		1.73	1.16	1.80	2.97
S.E. ±		0.38	0.26	0.40	0.66
% of total		31.9	25.2	42.9	

patients and the amount of total oestrogens was within normal limits but the relative value of oestradiol was high and highest in the case with hepatic coma.

In Case 3 the patient had repeated attacks of haematemesis and the liver-function tests were pathological. There was a slight increase of total oestrogen, but the oestradiol level was normal.

There was only one case (8) which showed a decreased amount of total oestrogens and this decrease included all the three fractions, although the liver-function tests were pathological.

Table IV

Urinary oestrogens (μg/24 hrs) and liver function tests

Case No.	Age	Oestrone E_1	Oestradiol E_2	Oestriol E_3	Total oestrogens	Serum bilirubin	Total plasma protein
1	20	4.26	8.25	2.03	14.54	0.3	6.6
2	22	7.14	4.34	9.47	20.59	0.7	7.6
3	22	5.27	3.55	7.25	16.07	0.6	6.8
4	35	4.39	8.01	6.29	18.69	0.9	8.2
5	26	5.07	5.69	0.42	11.18	1.2	6.2
6	32	5.27	7.39	1.45	14.11	1.6	5.9
7	22	1.61	10.92	0.89	13.42	3.2	6.2
8	25	2.79	2.01	3.17	8.15	0.8	5.5
9	30	6.51	9.60	5.38	21.49	0.8	6.7
10	27	8.18	15.86	2.73	26.75	0.6	6.8
Mean		5.07	7.56	3.91	16.54		
S.D. ±		2.18	3.78	3.03	5.57		
S.E. ±		0.69	1.19	0.96	1.73		
% of total		30.7	45.6	23.7			

Table V

Urinary oestrogens (μg/24 hrs) and liver function tests in a group

Case No.	Age	Oestrone E_1	Oestradiol E_2	Oestriol E_3	Total oestrogens	Serum bilirubin	Total plasma protein
1	28	9.86	10.28	11.61	38.75	0.6	7.2
2	22	8.67	10.02	22.82	41.01	0.5	6.7
3	27	7.89	17.37	28.50	53.76	1.1	6.3
4	25	3.11	15.74	16.32	35.17	0.4	7.3
5	35	9.14	9.26	33.32	51.72	1.6	6.8
6	33	27.21	5.91	51.37	84.49	0.6	7.1
7	36	12.11	16.71	33.73	62.55	2.2	5.6
8	19	12.61	6.77	32.20	51.58	0.8	0.1
9	26	7.88	16.54	27.62	52.04	0.7	7.2
10	24	14.17	14.31	13.08	41.56	0.5	6.3
Mean	—	11.26	12.29	27.06	50.61		
S.D. ±		6.29	4.33	11.83	15.26		
S.E. ±		1.99	1.37	3.51	4.83		
% of total		22.2	24.3	53.5			

in a group of male patients with bilharzial hepatic fibrosis

Alb./glob.	Liver tests		Enzymes				Clinical notes
	Thymol	$ZnSO_4$	Alk. phos.	S.G.O.T.	S.G.P.T.	Prothrombin per cent	
1.27	4	6	12	15	10	78	Ascites
0.13	3	8	8	12	12	76	—
0.92	6	11	11	27	28	65	Haematemesis
0.82	5	8	18	13	8	57	Hypotrichosis + impotence
0.72	6	10	17	23	18	63	Ascites + jaundice
0.54	2	10	23	34	21	48	Ascites + jaundice + gynaecomastia + palmar erythema + spider naevi
0.49	8	28	52	52	28	43	Ascites + jaundice + coma
0.69	6	11	16	11	9	52	— — —
0.85	7	12	7	32	22	53	Gynaecomastia + testicular softening
0.88	3	6	10	16	5	72	Gynaecomastia

of young female patients with bilharzial hepatic fibrosis

Alb./glob.	Liver tests		Enzymes				Clinical notes
	Thymol	$ZnSo_4$	Alk. phos.	S.G.O.T.	S.G.P.T.	Prothrombin per cent	
1.17	5	11	18	30	19	56	—
1.08	3	10	8	12	7	67	—
1.08	6	8	10	23	13	52	Ascites
1.22	8	13	3	10	8	76	—
0.49	12	14	16	34	12	44	Jaundice
0.78	7	12	28	82	32	45	—
0.58	8	10	14	34	21	43	Ascites + Jaundice
1.63	4	9	21	16	6	58	—
1.38	7	5	10	23	12	55	—
1.77	4	7	11	14	7	63	—

Table VI

Urinary oestrogens (μg/24 hrs) and liver function tests

Case No.	Age	Oestrone E_1	Oestradiol E_2	Oestriol E_3	Total oestrogens	Serum bilirubin	Total plasma protein
1	54	2.83	6.88	5.88	15.57	0.9	6.2
2	56	2.52	3.90	8.52	14.94	0.8	5.8
3	55	4.02	3.44	6.62	14.08	1.5	6.8
4	58	0.96	6.13	2.09	9.18	2.6	6.2
5	57	3.42	1.37	4.98	9.77	0.9	5.6
6	56	2.35	5.64	5.37	13.36	0.8	6.7
7	57	4.57	1.98	1.49	8.94	0.2	7.2
8	62	1.65	7.41	2.97	12.03	0.4	6.3
9	61	2.49	8.26	6.01	16.76	0.3	6.1
Mean		2.85	4.99	4.88	12.72		
S.D. ±		1.33	2.43	2.28	2.91		
S.E. ±		0.44	0.81	0.76	0.97		
% of total		22.4	39.2	38.4			

All the cases of the first group proved to be bilharzial hepatic fibrosis by liver biopsy.

Fig. 2 and Table V show the results in the group of young females with hepatosplenomegaly. Mean total oestrogens (50.61 μg/24 hrs) was slightly below the normal, but all cases were within normal limits. The oestriol fraction was significantly reduced ($P < 0.01$).

In Case 3 the patient had ascites; in Case 5, jaundice; and in Case 7, both. Liver function tests in these cases were pathological. The oestrogen level in these three cases did not differ from that in the cases without complications showing slightly to moderately affected liver function (Table V).

All these cases proved to be bilharzial hepatic fibrosis except Case 5 which was post-hepatic in nature.

No gynaecological complaint or endocrine manifestations were noted in these cases. Patients 2, 7, 8, 9 were single, the other six were married and had normal pregnancies and children.

Table VI and Figure 4 represent the results in the group of post-menopausal females with liver cirrhosis. A remarkable change in these was the significant increase in the oestradiol fraction ($P < 0.05$).

Five cases (1, 3, 4, 8 and 9) and especially 3, 4, 9 had advanced liver disease with poor liver-function tests (Table III). Of the five, three patients

in a group of post-menopausal women with liver cirrhosis

Alb./glob.	Liver tests		Enzymes				Clinical notes
	Thymol	$ZnSO_4$	Alk. phos.	S.G.O.T.	S.G.P.T.	Prothrombin per cent	
0.94	3	10	11	22	8	55	Ascites + palmar erythema + spider naevi
1.08	7	9	8	10	7	78	
0.88	7	12	31	44	24	55	Ascites + jaundice
0.85	8	9	21	30	15	51	Ascites + jaundice
0.61	2	6	26	53	41	45	—
1.38	4	6	12	16	10	67	—
1.32	6	5	6	7	7	58	—
1.13	7	12	7	15	12	62	Ascites + palmar erythema
0.92	10	11	20	26	21	57	Ascites

(1, 8, 9) had ascites and two (5, 4) jaundice and ascites. Two patients (1, 9) displayed palmar erythema. Spider naevi were noticed in Case 1. In all these cases liver biopsy revealed Laennec cirrhosis except Cases 1 and 5 which proved to be post-hepatitic.

Discussion

The results showed clearly that the oestradiol level as well as its per cent of the total urinary oestrogens were increased in liver disease. This may be due to the impaired capacity of the liver to transfer oestradiol into the less active oestrogens, as it was shown by SCHEDL [8] that the liver inactivates oestrogen. EL-MAHDY [3] found an increase in total urinary oestrogen accompanied by an increase in oestradiol and oestriol in male patients with uncomplicated bilharziasis, but a decrease of all the fractions in patients with hepatosplenic bilharziasis and ascites.

LEUTSCHER [5] found diminished libido and impotence in men with active hepatic cirrhosis and SZARVAS et al. [9] suggested that hypogonadism in cirrhotic patients was most likely the result of a primary testicular change.

The increase observed by us in oestradiol excretion in the patients with liver disease confirmed the findings of BROWN [1]. The decrease in

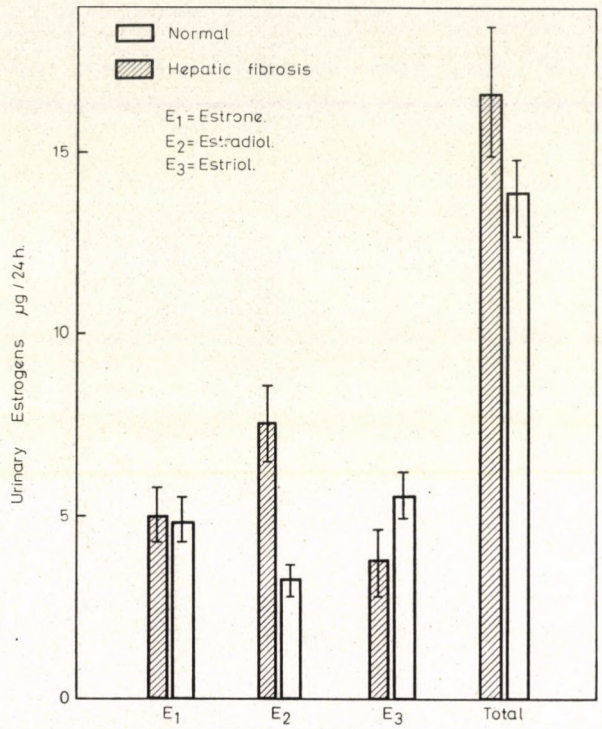

Fig. 1. Oestrogens µg/24 hrs in a group of normal Egyptian males and in a group of male patients with bilharzial hepatic fibrosis (Mean ± SD)

oestriol may be explained by the fact that oestrone is converted to hydroxy-oestrone, a precursor of oestriol, a step definitely impaired in patients with severe liver disease [3].

Thus, a direct correlation was found between the severity of the liver condition and the oestrogen picture. Fairly common was the increase in oestradiol, especially in males and post-menopausal women. Gynaecomastia or loss of axillary and pubic hair were not associated with the highest oestrogen values, so other factors also seem to be at play in the development of these symptoms.

Also, it might be due to the variable sensitivity of the cirrhotic patients' skin and mammary tissue to the hormones at issue that some cirrhotics have hypotrichosis and spider angiomata, while others do not show these manifestations. This also depends on the age being prepubertal or postpubertal when the hyperoestrogenemia occurs. If the maximum insult occurs before puberty the patient may suffer from severe hypogonadism and even infantilism. Hyperoestrogenemia may inhibit the anterior pituitary trophic hormones such as gonadotrophins and growth hormone. The condition may become worse when undernutrition and hypoproteinaemia are present.

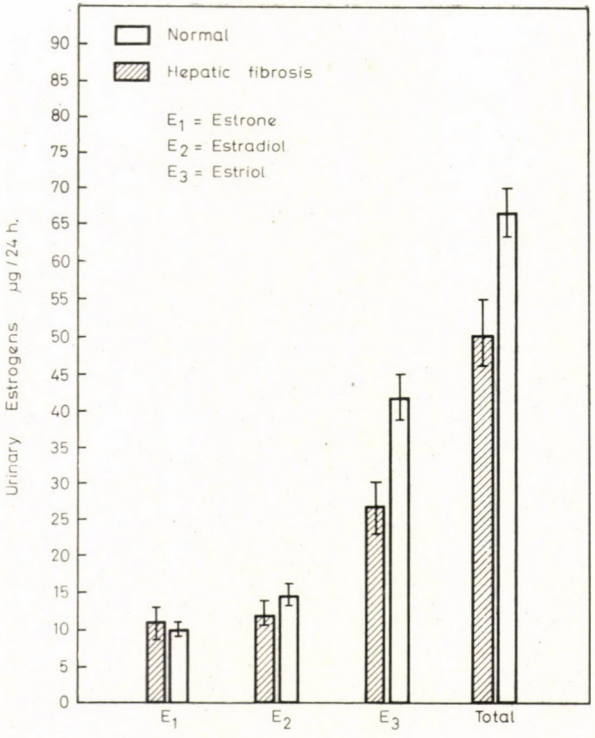

Fig. 2. Urinary oestrogens μg/24 hrs in a group of normal Egyptian young females and in a group of young female patients with bilharzial hepatic fibrosis (Mean $\pm$ SD)

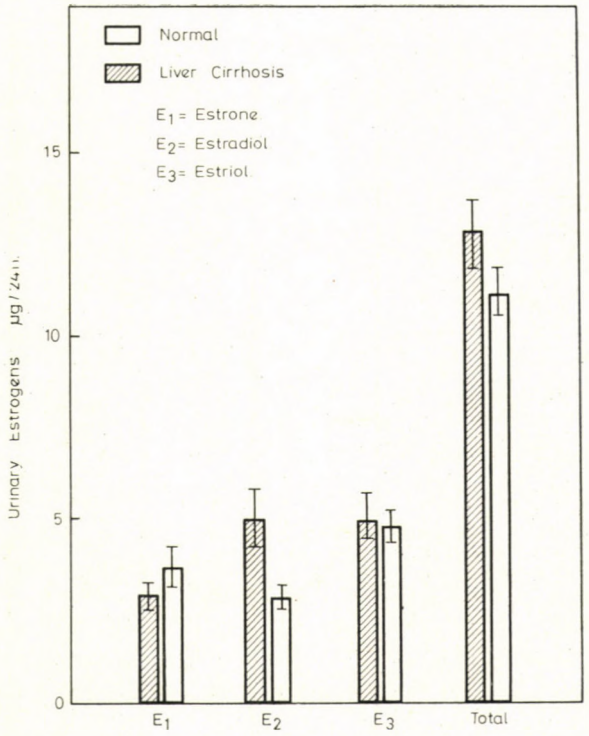

Fig. 3. Oestrogens μg/24 hr in a group of Egyptian postmenopausal women and a group of postmenopausal patients with liver cirrhosis (Mean $\pm$ SD)

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METABOLISM OF [4-^{14}C]DEHYDROEPIANDROSTERONE BY HUMAN SKIN IN VITRO

II. IN VITRO FORMATION OF WATER-SOLUBLE DEHYDROEPIANDROSTERONE SULPHATE AND ANDROST-5-ENE-3β,17β-DIOL-3-SULPHATE IN HUMAN ABDOMINAL SKIN

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Normal female and male abdominal skin slices were incubated with [4-^{14}C] dehydroepiandrosterone. Of the numerous radioactive metabolites formed, dehydroepiandrosterone sulphate, androst-5-ene-3β,17β-diol and androst-5-ene-3β,17β-diol-3-sulphate were isolated and identified by the reversed isotope-dilution method. It was confirmed that both female and male abdominal skin contain $\triangle^5$-3β-hydroxysteroid sulphokinase activity, which catalyzes the sulpho-conjugation of not only the dehydroepiandrosterone substrate, but also the androst-5-ene-3β,17β-diol formed during five-hour incubation. The significance of the water-soluble steroid sulphates formed in the skin is discussed.

The formation of water-soluble dehydroepiandrosterone sulphate (DHA-S) and androst-5-ene-3β,17β-diol-3-sulphate (Δ^5-diol-3-S) was first described by Gallegos and Berliner [1] who incubated male abdominal skin slices with [4-^{14}C]dehydroepiandrosterone (DHA). A few months later, the formation of DHA-S was confirmed in incubation experiments with female abdominal skin slices and [4-^{14}C]DHA [2]. These results permitted the conclusion that healthy male and female abdominal skin contains Δ^5-3β-hydroxysteroid sulphokinase (3β-HSS) activity, which catalyzes the formation from free DHA of water-soluble DHA-S and Δ^5-diol-3-S.

The importance of water-soluble steroid sulphate esters in human skin is not known. For this reason we have carried out incubation studies to determine whether female and male abdominal skin slices are equally capable of forming DHA-S and Δ^5-diol-3-S from [4-^{14}C]DHA. The present paper gives an account of the isolation and identification of these two sulphate esters.

Material and methods

Solvents. The organic solvents were of analytical purity, and were further purified by distillation.

Steroids. [4-^{14}C]DHA (specific activity; 57.1 mCi/mM) (The Radiochemical Centre, Amersham, England) was subjected to preliminary purification on an Al_2O_3-G thin layer in solvent system "D" (3). ATP, NAD^+ and $NADP^+$ were commercial products (Sigma Chemical Co., St. Louis, USA). The authentic steroids (DHA, DHA-S, Δ^5-diol, Δ^5-diol-3-S) were purified on thin layers in solvent systems corresponding to their polarities [4].

Skin samples. Normal female and male abdominal skin was obtained during opera-

tions for appendicitis. The "complete" skin samples, containing both dermis and epidermis, were purified from germicide and accompanying fatty tissue, and were then cut with scissors into 1—2-mm slices.

Incubation. 0.5 g of the female abdominal skin slices or 0.4 g samples of the male abdominal skin slices were incubated at 37°C for five hours in air with 1,084,296 dpm (0.4884 μCi, 8,553.793 pM) [4-^{14}C]DHA (spec. act.: 57.1 mCi/mM) in 5 ml Krebs—Ringer phosphate 200 mg per 100 ml glucose (KRPG) medium, at pH 7.3. The concentration of ATP, NAD^+ and $NADP^+$ in the incubation mixture was 10^{-3} M. Before incubation, the [4-^{14}C]DHA was dissolved in 0.2 ml propylene glycol. To avoid bacterial contamination, crystalline penicillin (1000 I. U./ml) and streptomycin sulphate (100 μg/ml) were added to the incubation medium. At the end of the incubation the reaction was stopped by the addition of 1 ml methanol, and the incubate was stored at —15°C.

As controls, samples of the same skin slices were inactivated by boiling in KRPG solution for ten minutes, and incubated with the substrate in KRPG medium in the presence of the cofactors.

In another control experiment only the [4-^{14}C]DHA substrate was incubated in the given medium.

Extraction. After the five-hour incubation, known amounts of "cold" DHA, DHA-S, Δ^5-diol and Δ^5-diol-3-S were added as carriers to each incubate, then the medium was poured off the skin slices.

The skin slices were washed first with 5 ml KRPG solution, and then with 5 ml 2 N ammonia solution, and the washings were added to the medium. The skin slices were next extracted with 4×5 ml methanol, and the extracts were evaporated to dryness in vacuum below 45°C.

The medium was poured onto the dry residue, the volume was made up to 20 ml with KRPG solution and then half-saturated with $(NH_4)_2SO_4$ (8 g/20 ml). The solution thus obtained was extracted with 4×20 ml ethyl acetate. The combined extracts, containing both free and water-soluble metabolites, were evaporated to dryness in vacuum below 45°C. 87—99% of the radioactivity of the medium was recovered during the extraction.

Chromatography

Column chromatography. The free and sulpho-conjugated metabolites were separated on a 1.5 g "Nymco" florisil column (60/100 mesh) (Floridin Co., Tallahassee, Florida, USA) [5]. 24 ml benzene was poured onto a 1.5 florisil column suspended in benzene, and the radioactive and "cold" steroids were then dissolved in 1 ml methanol and transferred to the column by Pasteur pipette. The methanolic solution was well mixed with the benzene, the solvent was allowed to drip through the column, and the whole operation was repeated. The 50-ml eluate thus obtained was combined: this was eluate 0. The column was next eluted with 3×20 ml 4% methanolic benzene (eluates 1—3), and then with 3×20 ml 20% methanolic benzene (eluates 4—6), 20 ml at a time. Eluates 0—2 from the column were combined into fraction I, and eluates 3—6 into fraction II. The radioactivity of fractions I and II was measured by a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3375). The efficiency of the apparatus for fraction I, containing the free steroids, was 86.5% for ^{14}C, while in the case of the sulpho-conjugated steroids (fraction II) it was 77.5%.

The free steroids (fraction I) were chromatographed on a 3 g Al_2O_3 column (E. Merck A. G., Darmstadt, Germany) (Brockmann activity III/IV), and the unchanged [4-^{14}C]DHA and the Δ^5-diol formed from the substrate were determined by the reversed isotope-dilution method described previously [6, 7].

The sulpho-conjugated steroids were isolated from fraction II and identified.

Thin-layer chromatography. 250 μ layers of either silica-gel-G (E. Merck A. G. nach Stahl) or Al_2O_3-G (E. Merck A. G. nach Stahl) are suitable for the separation of the free and the sulpho-conjugated steroids. The sulpho-conjugated steroids were separated as done by SARFATY and LIPSETT [8] using solvent system I (abs. ethanol — ethyl acetate — conc. NH_4OH (5 : 5 : 1, v/v/v) on Al_2O_3-G and silica gel-G [5].

The following thin-layer chromatographic (TLG) systems were applied for the separation of the free steroids;

Al_2O_3-G thin-layer

TLC "A": n-hexane — ethyl acetate — glacial acetic acid — abs. ethanol (210 : 30 : 2 : 0.5, v/v/v/v)

TLC "D": n-hexane — ethyl acetate — glacial acetic acid — abs. ethanol (140 : 100 : 0.5 : 2, v/v/v/v)

Silica gel-G thin layer

TLC "1 a": cyclohexane — ethyl acetate (60 : 40, v/v)

TLC "1 b": dichloromethane — ethyl acetate (4 : 1, v/v)

TLC "1 d": benzene — abs. ethanol (86 : 14, v/v)
TLC "2": benzene — ethyl acetate (3 : 2, v/v)
TLC "Lisboa-E": n-hexane — ethyl acetate — glacial acetic acid — abs. ethanol (13.5 : 72 : 10 : 4.5, v/v/v/v)

The positions of the radioactive spots on the thin-layer plates were determined with a Packard Radiochromatogram Scanner (Model 7201). The positions of the spots of the "cold" steroids were examined with a "Desaga" U. V. lamp (Model 131000) and iodine vapour. The steroids were extracted from the layer with methanol [5].

Determination of the amounts of steroids. The amounts of the "cold" DHA and DHA-S were determined spectrophotometrically via the Zimmermann reaction: the wavelengths used were 450, 510 and 570 nm, and the Allen equation was applied [9]. The amounts of Δ^5-diol and Δ^5-diol-3-S were determined after boiling for five minutes with concentrated sulphuric acid and measurement at 380, 410 and 440 nm [4, 6], with the help of the Allen correction equation.

Identification of DHA-S and Δ^5-diol-3-S. The two water-soluble sulpho-conjugated steroids were isolated from fraction II and identified. Fraction II was chromatographed in solvent system I, first on a silica gel-G layer, then on a Al_2O_3-G layer. In this way the sulpho-conjugated steroids were purified from any free steroids which may have been present, and were then solvolyzed. After solvolysis the Δ^5-diol and the DHA were separated on a silica-gel-G thin-layer, and then identified in the manner given in Table II. Specific activity was determined after every individual chromatographic step, and the DHA or Δ^5-diol was regarded as identified when its specific activity remained within a limiting error of 10% after two successive chromatographic steps. The amount of DHA-S or Δ^5-diol-3-S formed was calculated for 1 g wet skin tissue, and the conversion referred to the substrate was expressed in picomoles (pM), and then computed in percentage.

Preparation of steroid derivatives. Acetylation and hydrolysis were carried out according to BUSH [10]. Solvolysis was performed in accordance with TREIBER [11] as follows; 30 ml 1% perchloric acid solution in ether was added to the sulpho-conjugated steroids in a stoppered tube, and the mixture was left to stand at 37°C for 24 hours. The solvolyzed mixture was extracted with 1×5 ml 5 N NaOH, and then with 2×5 m distilled water. The ether was finally evaporated off to dryness below 45°C. Recovery could be carried out with a yield of 88—95%.

Results

The results given in Table I indicate that both normal female and male abdominal skin slices utilize [4-^{14}C]DHA under the experimental conditions

Table I

Control studies connected with steroid-sulphate esters formed in the course of incubation of human skin slices with [4-^{14}C]dehydroepiandrosterone

Cases studies	Incubation	Sulphate esters Total dpm/g skin/5 hr	Per cent	Dehydro-epiandrosterone sulphate Total dpm/g skin/5 hr	Per cent
1. normal female (24 years)	a) normal abdominal skin slices	62,957	5.81	49,732	4.59
	b) abdominal skin slices boiled for ten minutes	0	0.00	0	
	c) only [4-^{14}C]DHA substrate	0	0.00	0	
2. normal male (21 years)	a) normal abdominal skin slices	39,096	3.61	34,672	3.19
	b) abdominal skin slices boiled for ten minutes	0	0.00	0	
	c) only [4-^{14}C]DHA substrate	0	0.00	0	

Table II

Isolation and identification of unchanged substrate [4-^{14}C]dehydroepiandrosterone and androst-dehydroepi-

Identified steroid	"Cold" steroid μg	Chemical reaction	Derivative	TLC systems
Dehyroepiandrosterone+	317*	—	DHA	Syst. "D"
	210**	—	DHA	Syst. "2"
		Acetylation	DHA-Ac.	Syst. "1 a"
		Hydrolysis	DHA	Syst. "2"
Androst-5-ene-3β,17β-diol	213*	Acetylation	Δ^5-diol-diAc.	Syst. "A"
	213**	Acetylation	Δ^5-diol-diAc.	Syst. "1 b"
		Hydrolysis	Δ^5-diol	Syst. "Lisboa-E"

+ [4-^{14}C]dehydroepiandrosterone substrate (1,084,296 dpm = 0.4884 μCi, 8,553.793 pM)
* Amount of "cold" (carrier) steroid used for female skin slices
** Amount of "cold" (carrier) steroid used for male skin slices

described. If boiled skin slices were incubated with labelled DHA, neither sulphate ester nor DHA-S formation was observed. Similarly, metabolism could not be detected if only the substrate was incubated, without skin slices, under the given experimental conditions. These control experiments confirmed that the formation of sulphate esters is a result of the activity of the enzyme system in the skin.

Table III

Isolation and identification of dehydroepiandrosterone sulphate and Δ^5-diol-3-sulphate from nor-

Identified steroid	"Cold" steroid μg	Chemical reaction	Derivative	TLC systems
Sulphate esters	288*	—	Sulphate esters	Silica gel-G "I"
	216**	—	Sulphate esters	Al_2O_3-G "I"
Dehydroepiandrosterone		Solvolysis	Free DHA	Syst. "1 d"
sulphate		Acetylation	DHA-acetate	Syst. "1 a"
Δ^5-diol-3-sulphate	163+	Solvolysis	Free Δ^5-diol	Syst. "1 d"
		Acetylation	Δ^5-diol-diAc.	Syst. "1 a"
		—	Δ^5-diol-diAc.	Syst. "1 b"
		Hydrolysis	Δ^5-diol	Syst. "2"

* Amount of "cold" (carrier) steroid used for female skin slices
** Amount of "cold" (carrier) steroid used for male skin slices
+ Amount of "cold" (carrier) steroid used for female and male skin slices

5-ene-3β,17β-diol from normal female and male abdominal skin slices incubated with [4-^{14}C]-androsterone

Female skin (24 yr)			Male skin (21 yr)		
Specific activity dpm/μg	Incorpn/g/5 hr dpm	per cent	Specific activity dpm/μg	Incorpn/g/5 hr dpm	per cent
1,153.8			3,590.3		
1,243.7	432,030	39.85	2,584.8	533,022	49.16
1,371.2			2,491.6		
1,354.6			—		
123.30			54.26		
102.73	42,204	3.89	53.37	28,507	2.63
95.42			53.70		

The metabolites formed during the incubation of the normal female and normal male abdominal skin slices were separated into fractions I and II, i.e., into groups of free steroids and sulphate ester steroids. Table II presents the identification of the unchanged substrate and the resulting Δ^5-diol isolated from fraction I. A significant proportion of the [4-^{14}C]DHA was metabolized during the five-hour incubation of both the female and the male abdominal

mal female and male abdominal skin slices incubated with [4-^{14}C]dehydroepiandrosterone

R_f	Female skin (24 yr)				Male skin (21 yr)			
	Specific activity dpm/μg	Incorpn/g/5 hr			Specific activity dpm/μg	Incorpn/g/5 hr		
		total dpm	pM	per cent		total dpm	pM	per cent
0.41	108.28	62,957		5.81	73.03	39,096		3.61
0.31	110.32		496.65		71.78		308.42	
0.49	87.19	49,732		4.59	64.27	34,672		3.19
0.65	85.49		392.32		64.16		273.52	
0.35	—	—	—	—	—	—	—	—
0.63	14.96				18.21			
0.70	16.42	5,095	40.19	0.47	15.12	5,932	46.79	0.55
0.29	15.51				14.00			

skin slices. With the reversed isotope dilution method, 39.85% of the unchanged substrate was recovered from the incubate of female abdominal skin slices, and 49.16% in the case of the male abdominal skin.

The formation of Δ^5-diol could be shown from the in vitro incubates of both the female and the male abdominal skin slices. During the five-hour incubation the incorporation of radioactivity was 3.89% in the case of the female abdominal skin, and 2.63% for the male abdominal skin.

The proof of the formation of DHA-S and Δ^5-diol-3-S is given in Table III. It can be seen that during the five-hour incubation of the female and male abdominal skin slices, water-soluble sulphate esters were formed with significant radioactivity. In the case of the female abdominal skin slices the incorporation of radioactivity was 5.81% (496.65 pM), while for the male skin slices it was 3.61% (308.42 pM).

After solvolysis of the sulphate esters, chromatography in solvent system "1 d" on silica-gel-G thin-layer led to the separation of the radioactivity into two peaks. The radioactive peak exhibiting the greater movement ($R_f = 0.49$) ran together with DHA, and that with the lower movement ($R_f = 0.35$) ran together with Δ^5-diol. The constant specific activities of the steroids separated in various chromatographic systems and in the form of various derivatives confirmed that the steroids isolated from the sulphate ester fraction were identical with DHA-S and Δ^5-diol-3-S.

Table III shows that the majority of the radioactivity incorporated into the sulphate ester fraction had originated from the DHA-S. The DHA-S incorporation in the female abdominal skin was 4.59% (392.32 pM) and in the male abdominal skin 3.19% (273.52 pM). The formation of Δ^5-diol-3-S was much less. Radioactivity incorporation in the case of the female abdominal skin slices was 0.47% (40.19 pM), and for the male slices, 0.55% (46.79 pM).

Discussion

In addition to free DHA, DHA-S is an important secretory product of the adrenal cortex [12, 13, 14] and the ovary [15]. DHA-S circulates in peripheral blood in high concentration (10—550 μg/100 ml) [18, 19].

It is not known exactly what role the DHA-S plays in the transport, metabolism, or possible storage of DHA. Many authors regard it as an inactive metabolite of the weakly androgenic DHA [13, 14]. DHA-S can transform "directly" into Δ^5-diol-3-S, without the splitting-off of the sulphate group [20]. KIM and HERRMANN [21] incubated DHA-S with homogenizates of the vaginal mucosa of mature females, and the preputial and abdominal skin of infants. 5.7—14.2% of the substrate was converted to free DHA, and androst-4-ene-3,17-dione and testosterone too appeared in the incubate. DHA-S plays a role

in the regulation of the production of androgenic steroids by means of its "indirect" metabolism [3, 7, 21].

Besides the adrenal cortex [22], the gonads [23] too, presumably play a part in the formation of the Δ^5-diol. The latter can be found in peripheral blood in the form of mono- and disulphate esters [24]. The concentration of Δ^5-diol-3-S in the blood of males is much higher (28.7 μg/100 ml) than in the blood of females [25], and is about half the concentration of Δ^5-diol-3,17-disulphate [24].

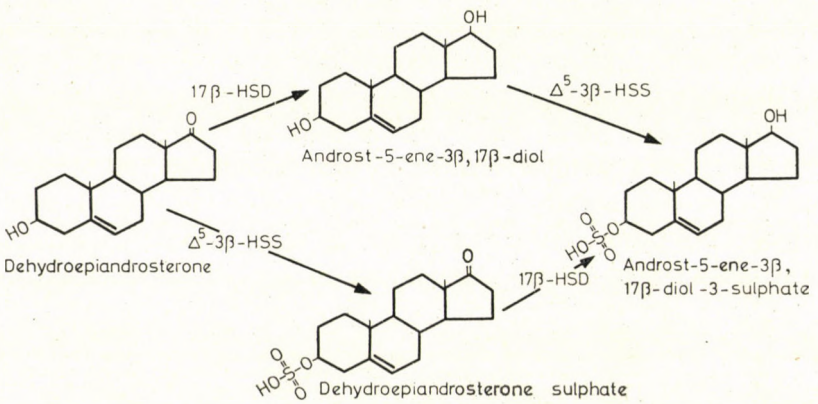

Fig. 1. Possible routes of [4-^{14}C]dehydroepiandrosterone-sulphate formation in normal human abdominal skin

Our studies support the results of previous authors [1, 26] in that in the course of incubation of female and male abdominal skin slices with [4-^{14}C]DHA, not only DHA-S but also Δ^5-diol-3-S is formed. Our results show that both the male and the female abdominal skin contains Δ^5-3β-HSS activity, which also catalyzes the sulpho-conjugation of the Δ^5-diol formed during the incubation with the DHA substrate. The possible routes for the formation of the Δ^5-diol-3-S in the skin are given in Fig. 1. The fact that free Δ^5-diol is formed in the incubate proves the presence of 17β-hydroxysteroid dehydrogenase activity in the skin, while the Δ^5-diol formed is converted to water-soluble Δ^5-diol-3-S with the participation of Δ^5-3β-HSS. As described by HAY and HODGINS [27] for the case of female axillary skin slices, this water-soluble steroid sulphate can also be formed by another pathway, the DHA-S pathway.

The steroid sulphokinase activities of the various human tissues have been studied by very many authors, and it has been shown that Δ^5-3β-HSS

enzyme is contained in the adrenal cortex, the liver, the ovary, the testes and the small intestinal mucosa [28, 29, 30, 31, 32]. The physiological significance of the Δ^5-3β-HSS activity detected in human skin has not been clarified. In our earlier studies it was shown that human axillary sweat contains a considerable amount of 17-Ks-S [33], the amount of which increases significantly on synthetic α^{1-28}-ACTH treatment [33].

Mention must be made of the studies by OERTEL and TREIBER [34], who isolated a whole series of radioactive androgenic steroids from sweat collected from the surface of the human trunk after intravenous injection of [7α-^{3}H]dehydroepiandrosterone-[^{35}S]sulphate-Na. Their results indicated that the overwhelming majority of the DHA, androsterone, etiocholanolone and Δ^5-diol detected in sweat could be isolated in free form, and lesser amounts in the sulphate-ester form. These studies confirmed the connection between the DHA-S level in peripheral blood and the amount of DHA-S secreted in sweat. We assume that the Δ^5-3β-HSS activity of the skin plays a role in the sulpho-conjugation of the androgenically active steroids entering the skin from the blood stream.

*

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DIAGNOSTIC VALUE OF THE RHEUMATOID ROSETTE

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The proportion of rheumatoid rosette-positive cases was examined in 57 patients with rheumatoid arthritis, 39 with some other autoimmune disease and 53 controls with other diseases. It was found that 1) 50% of the patients with rheumatoid arthritis were rosette-positive, as against 20% of those with other autoimmune diseases and barely 4% of the controls; 2) there was no direct correlation between the number of rosettes and the RF-serum level; 3) there was some correspondence between rosette positivity and the activity of the process.

Considering the fact that the RF as well as the rheumatoid rosette are connected with the anti-Ig-activity of the lymphoid cells, partly because RF may be present on the surface of the rosette-forming cells, partly because of the involvement of a direct cell-mediated Ig sensitivity, it is felt that the two diagnostic reactions are complementary, though their direct clinical significance remains questionable.

In 23 to 40% of the patients with rheumatoid arthritis (RA) no rheumatoid factor (RF) is demonstrable [1, 2]. This seronegativity might have the following explanation. 1) Formation of RF may be absent; 2) its amount may be too small to be detectable; 3) it may be present in a bound state which prevents its detection. All this has prompted a cellular approach to the study of the RF. The rheumatoid rosette test was described by Bach and Delbarre in 1968 [3]. O Rh-positive human erythrocytes coated with rabbit immunoglobulin were incubated with blood lymphocytes of patients with RA. The reaction was positive if a certain number of lymphocytes per thousand formed a binding with the coated erythrocytes in the form of rosettes. Extending the test to other diseases, the authors cited found that *a)* the rheumatoid rosette was positive in 70% of RA patients as against 5% of the controls; *b)* there was no direct correlation between the rheumatoid rosette and RF positivity, in other words, the rosette test may be positive in the absence of RF, and conversely.

The present study has been concerned with the proportion of rheumatoid rosette positive cases in our RA-patient material and with the correlations of the reaction with the other pertinent clinical and laboratory data.

Material and methods

Preparation of the lymphocyte suspension. 20 ml heparinized blood of the subject to be studied was allowed to sediment with 3% gelatin at 37°C for 45 minutes and the supernatant was filtered through a nylon wad column. The lymphocyte suspension was washed thrice with Parker's 199 medium containing 10% calf serum. The cell number was adjusted to 1.5×10^5 per ml.

Coating of the erythrocytes with immunoglobulin. Rabbits were immunized with 2×10^9 O Rh-positive human erythrocytes, administered intravenously on three occasions at 7 day intervals. This gave an anti-human rabbit haemagglutinin of a titre of approximately 1 : 1200. The lowest non-agglutinating dilution was determined. Since our haemagglutinin serum was known to produce no agglutination at twofold dilution, this dilution was adopted for the purposes of the present study. For coating, O Rh-positive human erythrocytes were washed three times with phosphate buffer and made up to a 2.5% suspension which was incubated at 37°C for 30 minutes with the antihuman rabbit haemagglutinin of the lowest non-agglutinating dilution. Then the suspension was washed three times and adjusted to 3 million cells per ml.

Rosette formation. 0.5 ml of the lymphocyte suspension and 0.5 ml of the Ig-coated erythrocyte suspension were centrifuged together at 600 r.p.m. for 10 min.

Reading. After careful resuspension in the nutrient medium, the number of rosette-forming cells/1000 lymphocytes was counted on a slide under a coverglass (Fig. 1).

Reproducibility. The procedure was repeated with the lymphocytes of 15 rosette-positive patients 3 to 7 times within 10 days. Apart from minor variations, the results were practically the same.

The results of the *Rose—Waaler-test* were regarded as positive when the titres were in excess of 1/32.

Patients. 149 patients were studied for the rheumatoid rosette. 57 had rheumatoid arthritis, 39 had other autoimmune diseases and 53 serving as controls had various other diseases. The second category comprised cases of SLE, polymyositis, dermatomyositis and scleroderma. In the control group there were cases of peptic ulcer, hypertensive disease, neurosis, and of other diseases unrelated to the first two groups.

In every case the clinical stage, the general condition, the ESR and the results of the Rose-test were put on record.

Results

Of the 53 controls 4 were seropositive (the Rose—Waaler-test giving a titre of 1/64 in 3 cases and of 1/128 in one), and 49 were RF-negative. In the negative group the rheumatoid rosette test was positive in two cases (in one of chronic nephritis and one of chronic pancreatitis).

The RF-positive patients of the control group were rosette-negative all throughout.

Table I

Results of the rheumatoid rosette test

Groups	Number of cases	≤ 6/1000	6–10/1000	> 10/1000
RF-positive RA	46	24	12	10
RF-negative RA	11	5	1	5
Other autoimmune diseases	39	31	3	5
Controls	53	51	1	1

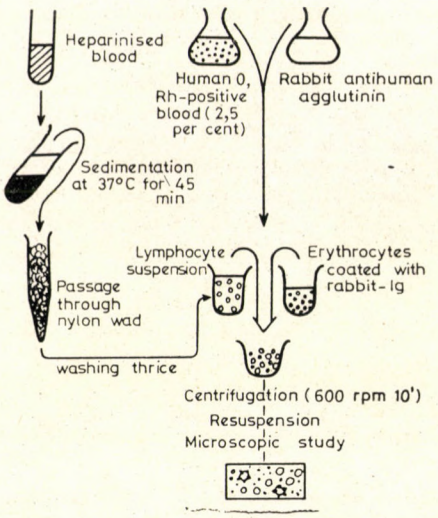

Fig. 1. The rheumatoid rosette test

The number of rheumatoid rosettes was in excess of 6/1000 in 28 out of 57 patients with RA, as against 8 out of 39 with other autoimmune diseases. 46 patients with RA were RF-positive, 11 were RF-negative. 22 out of 39 patients with other autoimmune diseases were RF-positive, 17 were RF-negative (Fig. 2, Table I, II).

Correlation between the number of rheumatoid rosettes and RF titres. 22 out of 46 RF-positive and 6 out of 11 RF negative RA patients were rosette-positive, the proportion of rheumatoid rosette positivity thus being 50% in both groups. In the group of other autoimmune diseases 5 out of 22 RF-positive cases (i.e., approximately 1/4 of the patients) and 3 out of 17 RF-

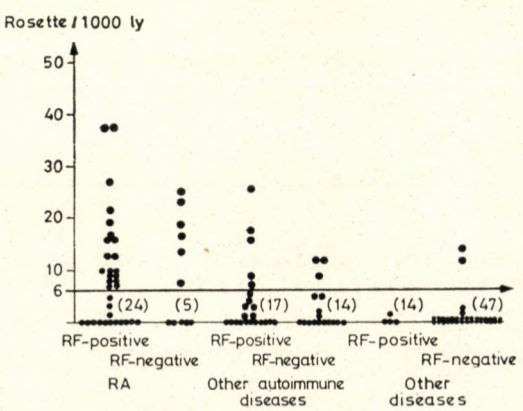

Fig. 2. Proportion of rheumatoid rosette positivity in the individual groups

Table II

Proportion of the results of the rosette test in the individual groups

Number of rosettes	Rheumatoid arthritis	Other autoimmun diseases	Control
< 4/1000 = neg.	26	24	51
4—6/1000 = uncertain	2	4	0
7—10/1000 = pos.	11	5	0
> 10/1000 = strongly pos.	15	3	2

negative cases (approximately 1/6 of the patients) were found rheumatoid rosette-positive.

On closer examination of the relationship between RF titre and rheumatoid rosette positivity it was found that the number of rheumatoid rosettes, so far from corresponding to the RF titres, shows an opposite tendency (Fig. 3). BACH et al. also note a significant negative correlation between the two parameters [4].

Correlation with clinical data.

1. Duration of disease. While positivity of the rosette test was less intensive in long-standing processes than in those of relatively recent onset, RF-positivity showed an inverse tendency (Fig. 4).

2. Activity of the process (arthralgia). A close correspondence has been found between arthralgia and rheumatoid rosette positivity, in opposition to RF which revealed no correlation of this kind (Fig. 5).

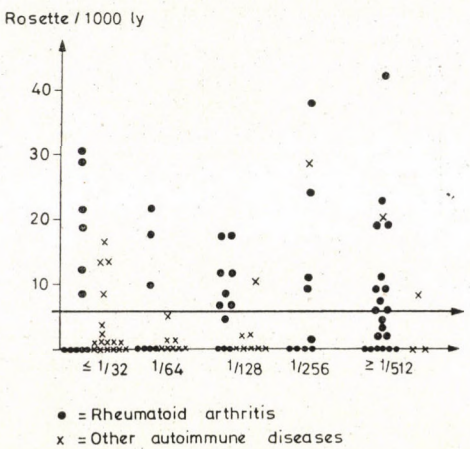

Fig. 3. Correlation between RF titre and the number of rheumatoid r[illegible]

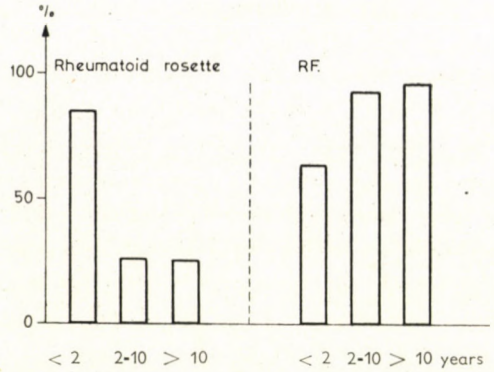

Fig. 4. Correlation of the duration of disease with rosette positivity and with RF titre

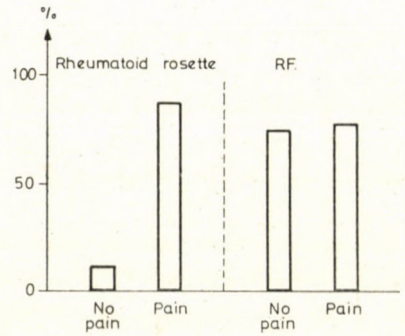

Fig. 5. Correlation of pain with rosette positivity and RF titre

3. Correlation with the sedimentation rate. With an ESR of more than 35 mm/hr the proportion of rosette-positive cases was significantly larger than below this ESR level (Table III).

Table III

Correlation between sedimentation rate and rheumatoid rosette positivity

	Number of cases	Rheumatoid rosette	
		negative	positive
$\leq$ 35 mm/hour	28	15	13
< 35 mm/hour	29	8	21

Discussion

On the evidence of this study, the rheumatoid rosette test proved positive in roughly 50% of the RF-positive and of the RF-negative group alike. Our figures are thus lower than those reported by BACH et al. who found the test positive in 70% of their cases. This may have the following causes: *a)* our cases were smaller in number; *b)* the majority of our patients were on immunosuppressive treatment (steroids, in some cases cytostatic drugs) which interferes with the formation of rheumatoid rosettes; *c)* there might have been minor differences in the technique, such as pH, incubation time, diameter of the tubes used for incubation, etc.

The rosette-forming lymphocytes are assumed to be either RF-producing, or rabbit-Ig(antigen)-sensitive cells. Experimental evidence suggests, however, that the cells responsible for the formation of the rheumatoid rosette are not of the same type [5, 6], part of them belonging to the antibody-forming lymphocytes of type "B" and the other to the antigen-sensitive lymphocytes of type "T." The observation that nontoxic concentrations of azathioprine, known to affect primarily the cells of type "T," are but incompletely suppressive to rosette formation [6], also seems to be consistent with a possible involvement of two different lymphocyte populations [6].

Previous treatment of the lymphocytes with anti-human immunoglobulin has been found to inhibit rheumatoid rosette formation [4, 7]. On the evidence of morphological studies the majority of the rosette-forming cells are small lymphocytes [8].

Monocytes are also capable of forming non-specific rosettes, there is, however, no difficulty in identifying these cells light-microscopically and thus in eliminating them in the course of counting.

RF positivity and rosette positivity. No direct correlation has been found between RF positivity and the number of rheumatoid rosettes. This admits of the following interpretations.

a) While rosette formation is the result of the proliferation of anti-gamma G-specific lymphocytes and of their release to the periphery, the RF level is closely related to the secretory activity of specific plasmoid lymphocytes. An increase in the number of rosettes representing the cellular immune phenomena ensuing earlier in time, precedes the demonstrability of the RF. It is moreover possible that the antigenic stimulus elicits merely a cell-mediated response with no elevation of the antibody level (in the case of RA, of the RF level). Under experimental conditions, the rheumatoid rosette phenomenon is demonstrable a few days earlier than the RF [4], on the other hand, the RF is still present in blood when the rosettes are no longer demonstrable. *b)* It is possible that RF-producing cells of type "B" account only for a certain part of the rosette-forming population, the other part con-

sisting of antigen-sensitive "T" cells. *c)* Finally, as presumed by BROWN and EPSTEIN [8] excessive amounts of RF antibody may well be suppressive to the rosette-forming lymphocytes.

RF positivity and rosette negativity. 24 out of 46 RF-positive cases of RA were rosette-negative. The majority of these patients were in remission or under treatment.

RF negativity and rosette positivity. 6 out of 11 RF-negative cases of RA were rosette-positive. The lack of a direct correlation between RF and the rheumatoid rosette has been discussed earlier in this paper.

RF negativity and rosette negativity. RF negativity and rosette negativity were coupled in 7 cases of RA, all of minor severity, having been in complete remission for a fairly long time.

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EFFECT OF HEPARIN ON THE CONCENTRATION OF INSULIN IN SERUM AND PLASMA

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By using the immunoreactive double antibody assay of Hales and Randle in heparin plasma and heparinized serum, a lower insulin concentration was observed than in serum without heparin. In the radioimmunoreactive single antibody method of Ziegler et al., heparin failed to influence the amount of insulin. It has been concluded that the effect of heparin is connected with the presence of the second — anti-gammaglobulin — antibody.

A high serum insulin concentration may occur as a consequence of the second antibody inhibiting the effect of complement as well as of the cross-reaction between the second antibody and human gammaglobulin. Because of the favourable effect of heparin on the anticomplement and the optimum precipitation zone it is advisable to determine the insulin value in heparin plasma by the radioimmune double antibody assay. A blood heparin concentration of 1.0 IU/ml proved to be sufficient for ensuring a favourable effect.

The relative simplicity of radioimmune insulin determination offers a great help in diagnostics and research. Data on the concentration of normal insulin in human serum and plasma are far from uniform [14]. The explanation of the differences lies in the fact that some authors determined the insulin in serum, others in plasma prepared in different ways, and there are even data on "insulin μU/ml" (serum? plasma?). Further differences are arising from the variety of the methods applied, the effect of chemicals used for preparation of the plasma, and, finally, from individual variations (Fig. 1).

The present study was aimed at clarifying two points, *viz.*

1) Is there any difference between the serum and plasma insulin levels in the same subject?

2) Does heparin added to the serum in vitro influence the insulin level?

Material and methods

1. *Insulin concentration in serum and heparin plasma.* Fasting blood samples were taken. The plasma heparin concentration was 15 μg/ml (Heparin Richter, 1 mg = 134.6 IU). 75 μg of heparin was given to 5 ml of blood in a test tube containing 0.1 ml of physiological NaCl solution.

2. *Effect of heparin in vitro on the serum-insulin concentration.*

Heparin dissolved in physiological NaCl solution at a concentration of 1, 5, 500 μg/ml was added to serum at a ratio of 0.1 : 0.9. In the determination of the serum-insulin concentration, instead of heparin solution 0.1 ml of physiological NaCl solution was applied in order to ensure standard conditions.

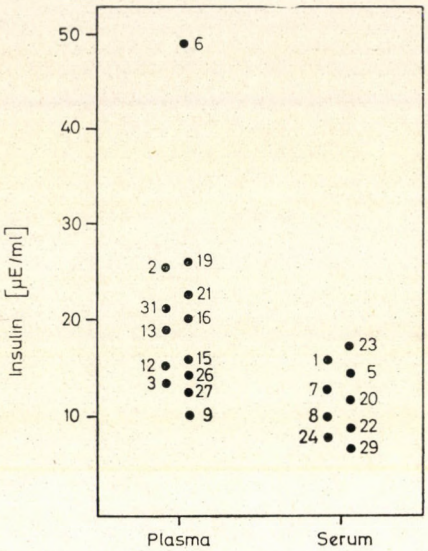

Fig. 1. Fasting insulin level of healthy subjects determined by different immune assay on the basis of 22 reports. The numbers show the literary references (after HENDERSON)

3. *Insulin concentration in serum, heparin plasma, EDTA plasma and the effect of heparin in vitro on the insulin level.*

On preparing EDTA plasma, the concentration of EDTA was 0.75 mg/ml in the blood, and the concentration of heparin was 15 μg/ml in the heparin plasma. 1, 5 or 500 μg/ml of heparin was added in vitro to the serum, heparin plasma and EDTA plasma.

The examinations were performed on healthy subjects and patients with hyperinsulinism, prediabetes, and diabetes. The high standard error was due to the simultaneous occurrence of normal and pathological high values. Insulin was determined by the method of HALES and RANDLE [11] as well as that of HADING [10] as modified by ZIEGLER et al. [30]. In groups 2 and 3 both methods were used in parallel.

Significance was estimated by the *t*-test.

Results

1. The concentration of insulin was lower in heparin plasma than in serum. The difference was not significant (Table I).

2. *A)* Heparin given to the serum in vitro decreased significantly the value for insulin concentration, whereas a further dose of heparin added to heparin plasma failed to cause any change (Table II, Fig. 2).

B) The insulin level was determined in serum and plasma of the same person in 10 cases by two different immunoreactive methods. Two antibodies (antiinsulin- and antigammaglobulin-antibody) were used by HALES and RANDLE [11] (method *A*), and only one (antiinsulin-antibody) by ZIEGLER et al. [30] (method *B*).

Table I

Insulin concentration in serum and plasma estimated by the Hales—Randle method

	Serum	Heparin plasma
M	34.8	21.7
SEM	± 4.3	± 2.7
p		n.s.
n	30	30

M = mean value μE/ml
SEM = standard error of mean
p = significance level
n = number of experiments
n. s. = not significant

When heparin was added to the serum, lower insulin values were obtained by method *A*) (Table III/A, Fig. 3/A). The change was not significant because of the great standard error and the relatively few examinations. Heparin added to heparin plasma in vitro failed to cause any change. Heparin did not affect the insulin value if the determination was carried out by method *B* (Table II/B, Fig. 3/B).

3. In group 3, insulin was determined in serum, heparin plasma and EDTA plasma of the same blood after adding heparin at a concentration of 1, 5 or 500 μg/ml.

Table II

Insulin concentration in serum and plasma after the addition 1, 5, 500 μg/ml heparin in vitro, estimated by the Hales—Randle method

Heparin in vitro μg/ml	0	1	5	500
	Serum			
M	48.4	29.1	30.8	34.1
SEM	± 3.4	± 3.4	± 3.2	± 3.3
p		< 1%	< 1%	< 1%
n	24	24	24	24
	Heparin plasma			
M	36.5	31.6	32.8	30.4
SEM	± 3.0	± 3.1	± 3.0	± 3.2
p		n.s.	n.s.	n.s.
n	24	24	24	24

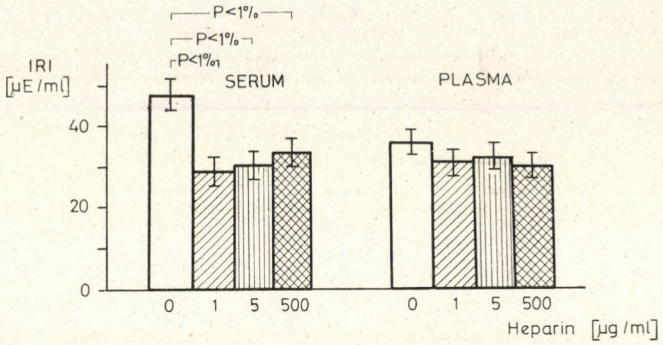

Fig. 2. Insulin level in serum and plasma after the addition of heparin at a concentration of 1, 5 or 500 μg/ml in vitro, estimated by the HALES—RANDLE method

In method *A*, addition of heparin to the serum resulted in lower insulin values, while in heparin plasma it failed to cause any change. In the case of EDTA plasma the change was not unequivocal. The insulin values measured in EDTA plasma were higher than in serum or in heparin plasma (Table IV/a, Fig. 4/A).

In method *B*, when a heparin concentration of 500 μg/ml was used, an increase in the insulin level was observed, without any other changes. The insulin level in serum, heparin plasma and EDTA plasma was practically identical when assayed by method *B* (Table IV/B, Fig. 4/B).

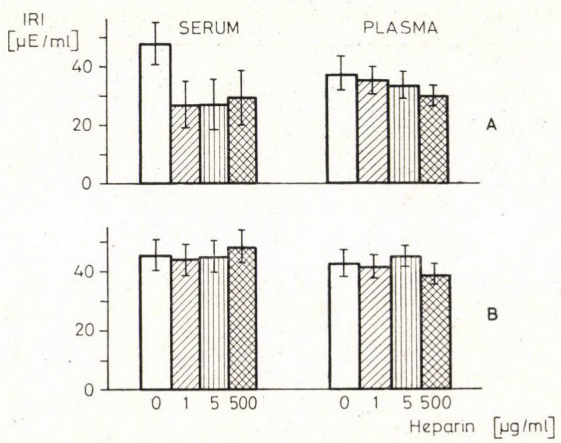

Fig. 3. Insulin level in serum and plasma after the addition of heparin at a concentration of 1, 5 or 500 μg/ml in vitro, as estimated by the methods of HALES—RANDLE (A) and ZIEGLER et al. (B)

Table III/A

Insulin concentration in serum and plasma after the addition 1, 5, 500 μg/ml heparin in vitro, estimated by the Hales—Randle method

Heparin in vitro μg/ml	0	1	5	500
Serum				
M	48.2	26.9	26.9	29.6
SEM	± 7.4	± 7.9	± 8.5	± 9.0
p		n.s.	n.s.	n.s.
n	10	10	10	10
Heparin plasma				
M	33.9	35.4	33.9	30.0
SEM	± 5.5	± 4.7	± 4.4	± 3.6
p		n.s.	n.s.	n.s.
n	10	10	10	10

Table III/B

Insulin concentration in serum and plasma after the addition 1, 5, 500 μg/ml heparin in vitro, estimated by the method of Ziegler et al.

Heparin in vitro μg/ml	0	1	5	500
Serum				
M	45.7	43.7	45.1	48.4
SEM	± 5.2	± 5.2	± 5.0	± 4.8
p		n.s.	n.s.	n.s.
n	10	10	10	10
Heparin plasma				
M	42.9	41.7	45.4	38.6
SEM	± 4.7	± 3.7	± 3.5	± 3.7
p		n.s.	n.s.	n.s.
n	10	10	10	10

Table IV/A

Insulin concentration in serum and plasma after the addition 1, 5, 500 μg/ml heparin in vitro, estimated by the method of Ziegler et al.

Heparin in vitro μg/ml	0	1	5	500
Serum				
M	34.8	19.4	29.0	27.0
SEM	± 14.2	± 9.0	± 9.6	± 11.8
p		n.s.	n.s.	n.s.
n	5	5	5	5
Heparin plasma				
M	24.4	26.0	23.0	25.8
SEM	± 11.5	± 13.1	± 12.3	± 10.8
p		n.s.	n.s.	n.s.
n	5	5	5	5
EDTA plasma				
M	47.4	58.4	46.0	56.0
SEM	± 17.6	± 23.3	± 15.9	± 17.6
p		n.s.	n.s.	n.s.
n	5	5	5	5

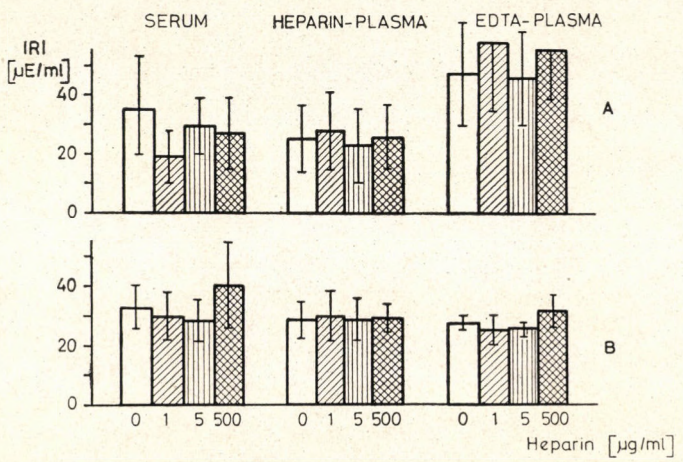

Fig. 4. **Insulin level in serum and plasma after the addition of heparin at a concentration of 1, 5 or 500 μg/ml in vitro, as estimated by the method of HALES—RANDLE (A) and ZIEGLER et al. (B)**

Table IV/B

Insulin concentration in serum and plasma after the addition 1, 5, 500 μ/ml heparin in vitro, estimated by the method of Ziegler et al.

Heparin in vitro μg/ml	0	1	5	500
Serum				
M	33.2	29.8	28.4	40.6
SEM	± 7.7	± 7.9	± 6.8	± 14.4
p		n.s.	n.s.	n.s.
n	5	5	5	5
Heparin plasma				
M	28.8	30.0	29.2	29.6
SEM	± 6.0	± 8.3	± 7.0	± 4.4
p		n.s.	n.s.	n.s.
n	5	5	5	5
EDTA plasma				
M	28.2	25.6	25.8	32.2
SEM	± 2.3	± 4.8	± 2.3	± 5.4
p		n.s.	n.s.	n.s.
n	5	5	5	5

Discussion

Data in the literature are not uniform concerning the changes of insulin concentration in plasma and serum as well as in their direction (+ or —) under the effect of heparin added to the serum or full blood in vitro in the course of the determination.

According to HENDERSON [14] the addition of heparin to blood resulted in a higher insulin value, and he suggested to determine the insulin not in heparin plasma but in serum. We ourselves obtained a lower insulin value in heparin plasma and in serum heparinized in vitro than in serum, in agreement with the data of a number of reports [4, 18, 24, 25, 28].

No negative parallelism was found between the change of the serum insulin concentration and the increase of the dose of heparin while a negative parallelism was observed by SOELDNER and SLONE [24] between the amount of heparin and the value for insulin. They observed that the plasma obtained by mixing blood with a known amount of heparin has the same insulin concentration as the serum containing the same amount of heparin.

Heparin can affect the insulin level by several mechanisms. In part of our studies — when heparin was added to the serum — the role of coagulation could certainly be excluded.

The insulin value was not influenced by the addition of heparin to the serum or blood if the method of ZIEGLER et al. [30] was used for the estimation of insulin. Since in this method only one antibody — an antiinsulin antibody — is applied, the effect of heparin must be connected with the presence of the second, the so-called precipitating, antibody.

According to HALES and RANDLE [12], the cross-reaction between human gammaglobulin and the percipitating antiserum may cause artificially high insulin values. This can be reduced by ensuring the optimum concentration of precipitating antiserum. The studies by MORGAN et al. [17] indicate that the artificially high serum-insulin values are not due to the cross-reaction but to the complement.

The application of heparin is favourable and necessary in the course of insulin determination by the double antibody assay as, in view of its anticomplementary effect, it accelerates the reaction of the second antibody, widens the zone of optimum precipitation, and in this way eliminates the artificially high insulin of the serum samples [4, 28]. This is why it is reasonable to determine insulin in heparin plasma.

To determine and standardize the optimum dose of heparin is of the greatest importance. As heparin slightly inhibits the reaction of the first antibody, according to WELBORN and FRASER [28], it is desirable to apply the optimum dose of heparin. WELBORN and FRASER [28] used heparin at a concentration of 25 IU/tube, BRUNFELDT and JORGENSEN [4] 125 IU/ml. According to our investigations, even 0.15—0.7 IU/ml of heparin (Richter) in plasma can ensure the favourable effect of heparin in the course of the determination. In the determination of the heparin dose, it must, however, be remembered that the anticomplementary effects of several heparin preparations are different and they do not show a parallelism with the anticoagulant activity.

Conclusions

1. For insulin determination it is more suitable to apply the radioimmune double antibody assay in heparin plasma than in serum or plasma obtained in different ways.

2. It is desirable to standardize the heparin dose and to use the same product for the preparation of heparin plasma. A blood concentration of 1.0 IU/ml of heparin is sufficient to ensure the optimum heparin effect.

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CYTOTOXICITY OF HUMAN LYMPHOCYTES IN TISSUE CULTURE

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A new ^{51}Cr radioisotope procedure for the study of the cytotoxicity of human lymphocytes has been developed. Monolayers formed by labelled amnionic cell cultures are inhibited in their adhesion to glass surfaces by cytoaggressive lymphocytes. The resulting difference in activity is measurable. The procedure is rapid, it permits 24-hr readings and the results are reproducible.

The cytotoxic effect of lymphocytes of subjects with autoimmune disease and of controls have been studied by this procedure. The lymphocytes of subjects being in active periods of autoimmune disease and receiving no immunosuppressive treatment caused a significant destruction of cultured human amnionic cells. The cytotoxic effect is far less extensive in periods of remission even in untreated cases.

Immunosuppressive treatment results in a significant attenuation of target-cell destruction.

The procedure is believed to lend itself to the assessment of the degree of cell-mediated immunoagression and of the efficacy of immunosuppressive treatment in autoimmune and in heteroimmune reactions (e.g. homograft-rejection). In acute experiments, administration of 6-mercaptopurine in a single 100 mg dose was followed by a total suppression of the cytotoxic activity of lymphocytes obtained from a patient with rheumatoid arthritis and three hours later their citotoxicity was far less intensive than before the administration of the immunosuppressive agent.

The cytotoxic (cytolytic) effect of lymphoid cells has been given much attention in recent years, this effect being closely involved in homograft-rejection and in tumour immunity alike. The mechanism of the effect is far more difficult to study in vivo than in model experiments.

The present study was concerned with the elaboration of a suitable procedure for the assessment of the target-cell aggressivity of lymphocytes and sought answers to the following questions.

Are the lymphocytes of subjects with autoimmune disease more toxic to homologous (allogeneic) cell cultures than are lymphoid cells of other patients or of healthy subjects?

Is the activity of autoimmune disease or, conversely, its remission produced by treatment, apt to modify the cytotoxic aggressivity of lymphocytes?

How far is the aggressivity of lymphocytes on the target cells affected by immunosuppressive agents in acute experiments?

Material and methods

The procedure is based on the principle that intact cells cling to glass surfaces whereas affected cells fail to do so.

1) *Lymphocytes.* Ten ml heparinized venous blood was poured onto a cotton column (400 mg cotton in a 10 ml syringe) and left to stand, thus allowing the granulocytes to adhere to the cotton threads. The column was then washed with 30 ml Parker's 199 medium, the washing fluid was allowed to sediment with 1/3 part of 3% gelatin at room temperature for 1 hour. The supernatant was centrifuged at 500 r.p.m. for 10 min, then washed three times with Parker's 199. After the second washing the residual erythrocytes were removed with distilled water (4 ml for 20 sec), then the cell supension was made up with the medium to 30 ml. The lymphocyte suspension had to meet the standard of 90% purity which was checked by cell counting at the end of separation.

2) The *target cells* were human amnionic cells (from the strain maintained at the University Microbiological Institute. Debrecen, for 2 years). From the cell strain submitted to weekly passages a suspension of 2 million/ml was prepared on the occasion of a passage and 25 μci ^{51}Cr chromate was added to it. The mixture was incubated at 37°C for 1 hour, then the unbound chromate was removed by washing with Parker's 199. The labelling proved to be of 30 to 40% efficiency. We ignore to which particular cell structure the isotope is linked and we have not found any indication in the literature on this point.

3) *Mixed lymphocyte target-cell culture.* Portions of 50,000 amniotic cells were placed in Wassermann-tubes and the lymphocyte suspension was added in 10, 50 and 100fold amounts. The mixed cell suspensions were made up with Parker's 199 to 1.5 ml. Parallels of 2 to 4 tubes were handled in the same manner. The tubes were placed in a frame in an inclined position forming an angle of 8° with the horizontal line and incubated at 37°C for 20 hours.

4) *Evaluation.* After incubation the radioactivity of each tube was measured, the parallel values were averaged. In this manner was obtained the total activity. The medium was poured off, the tubes were washed twice with physiological saline, then the activity of the cells still adhering to the wall of the glass tubes were measured again. The values thus obtained were expressed in per cent of the total activity. It was noted in preliminary experiments that a failure of the cells to adhere to the surface was a highly sensitive indicator of cellular lesion. In fact, the cells are unable to cling to the wall even before their complete destruction.

Patients

The blood lymphocytes of 30 patients with autoimmune disease were studied. There were 6 males, 24 females, 12 of the patients being in an active, 18 in an inactive period. The lymphocytes of 10 other patients (3 males, 7 females) and of 7 healthy subjects (2 males, 5 females) were also studied.

Results

I. Fig. 1 represents the adhesion tendency against time of the amnionic cells. In the presence of aggressive lymphocytes the ^{51}Cr labelled amnionic cells attached poorly to the wall (Fig. 1).

The cytotoxicity of lymphocytes separated in active periods of autoimmune disease was greatly enhanced by increasing the amnionic cell/lymphocyte ratio of 1 : 10 to 1 : 50 or to 1 : 100. Fig. 2 shows the decrease of activity exhibited by the amnionic cells adhering to the wall, in other words, the intensification of cytotoxicity, parallel with the increase in the number of cytoaggressive lymphocytes (Fig. 2).

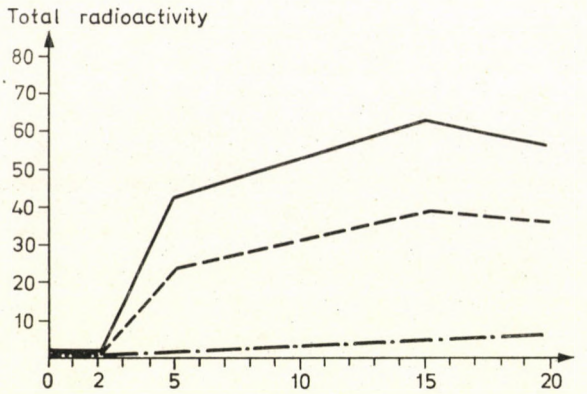

Fig. 1. Inhibition of adhesion of ^{51}Cr-labelled human amnionic cells by human blood lymphocytes
——— = Amnionic cells only
- - - - - - = Amnionic cells: Lymphocytes = 1 : 10
—.—.— = Amnionic cells: Lymphocytes = 1 : 50
Lymphocytes of T. J., 46 year, ♀, with rheumatoid arthritis. The inhibitory effect on the adhesion of target cells increases with the proportion of lymphocytes

II. It has been found in agreement with published evidence [2, 10, 11, 13] that lymphocytes of subjects with autoimmune disease (systemic lupus erythematosus, rheumatoid arthritis, dermatomyositis) are more toxic to cells of homologous cell cultures than are those of controls. The intensity of this cytotoxicity is related to the activity of the disease. However, consideration must be given to the fact that immunosuppressants, whether steroids or cytotoxic drugs, are apt to modify the cytoaggressivity of lymphocytes in various ways.

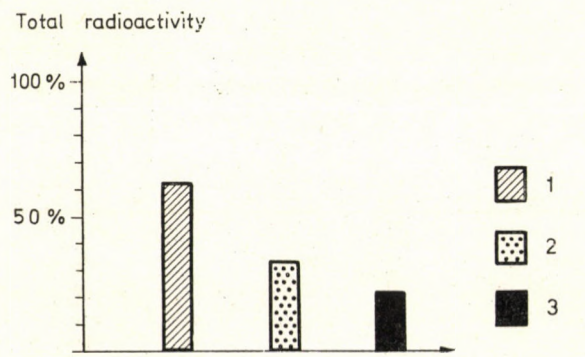

Fig. 2. Sz. Z., female patient with rheumatoid arthritis. Radioactivity of the adhering cells measured at 37°C after 24 hours incubation is expressed in per cent of total radioactivity: 1. Amnionic cells alone as controls. 2. Amnionic cells: lymphocytes 1 : 10. 3. Amnionic cells: lymphocytes 1 : 50

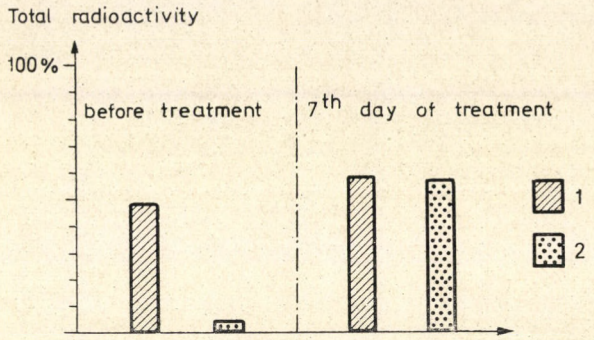

Fig. 3. Cz. G., female patient with SLE. Lymphocytotoxicity before treatment with 6-MP in daily doses of 100 mg and on the 7th day of treatment. Activity of the adhering amnionic cells is expressed in per cent of total activity: 1. Amnionic cells alone as controls. 2. Amnionic cells : lymphocytes 1 : 10

III. The influence of immunosuppressive therapy in autoimmune disease on the cytotoxicity of lymphocytes has been studied by comparison with the intensity of cytotoxic effect found before treatment. In Fig. 3, the data of a patient with systemic lupus erythematosus, in Fig. 4, of one with rheumatoid arthritis are seen before treatment with mercaptopurine (6-MP) and after 7-and 14-day treatment. Immunosuppressive treatment has thus been found to result in total suppression of the cytotoxicity of the lymphocytes, as reflected by a normal adhesion of amnionic cells similarly to those of the control tubes. These findings are consistent with the favourable clinical response, in particular as concerns arthralgia, temperature and ESR (Figs 3 and 4).

IV. Heparinized blood was taken from patients with active rheumatoid arthritis having had no immunosuppressive treatment, then 100 mg 6-MP

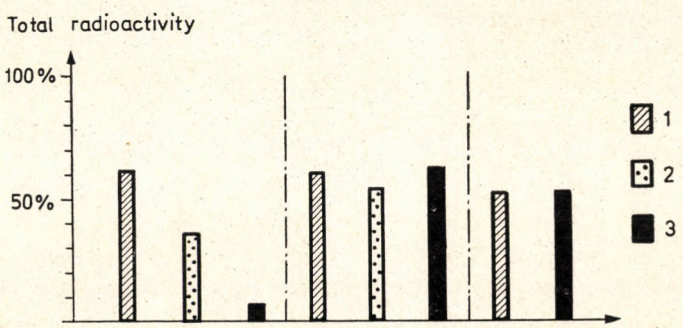

Fig. 4. Lymphocytotoxicity under the influence of 6-MP-treatment. K. J., female patient with rheumatoid arthritis. Lymphocytotoxicity before treatment with 6-MP in daily doses of 100 mg and at 7 and 14 days of treatment. Radioactivity of the adhering cells is expressed in per cent of total radioactivity: 1. Amnionic cells alone as controls. 2. Amnionic cells: lymphocytes 1 : 10. 3. Amnionic cells: lymphocytes 1 : 50

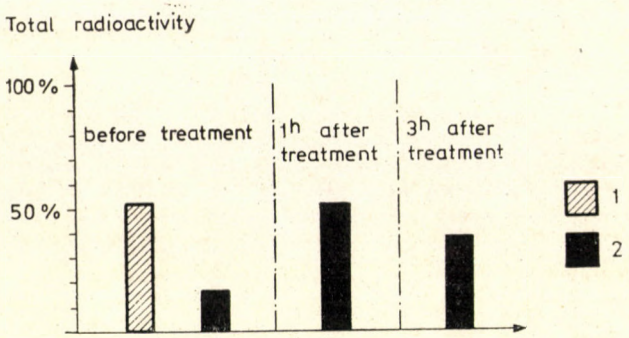

Fig. 5. Cytotoxicity of lymphocytes separated 1 and 3 hours after oral ingestion of 100 mg 6-MP. Activity of the adhering amnionic cells measured after 20 hours incubation is expressed in per cent of total radioactivity: 1. Amnionic cells alone as controls. 2. Amnionic cells: lymphocytes 1 : 50

was administered by mouth and blood was taken again at 1 and 3 hours. The lymphocytes were separated and their cytotoxicity was examined by the procedure described above (Fig. 5). It can be seen in Fig. 5 that, while before administration of the immunosuppressive agent the patient's lymphocytes were definitely toxic to the amnionic cells, only a minority of them having been capable of clinging to the wall, by the end of the first hour after ingestion of the drug the lymphocytes have practically lost their cytotoxicity, and at the end of the third hour they have not yet regained their full cytostatic activity.

Discussion

Studies concerning the destructive effect of lymphocytes on cells and tissues in vitro date scarcely ten years back. BRAUNSTEINER et al. [1963], examining the effect of lymphocytes on amnionic cell cultures found a contact aggregation of the majority of lymphocytes to the cells within the first three hours and an intensive amnionic cell destruction at the end of 24 hours. Lymphocytes derived from lymph nodes of healthy subjects exhibited no destructive effect of this kind. HOLM et al. [1964] studied the cytotoxic effect of phytohaemagglutinin-stimulated lymphoid cells of a normal, nonsensitized individual in allogeneic and xenogeneic systems. The target cells were labelled with ^{14}C-thymidine and the extent of cell desctruction was expressed by the amount of thymidine being released as a result of cell destruction. Cr-isotope labelling with measurement of Cr release, has, however, been more generally adopted [4, 5]. WILSON [1965] assessed the intensity of homologous target-cell destruction by modifying the incubation time and the amount of lympho-

cytes. He found that in addition to the duration of incubation the number of lymphocytes added to the culture was also essential. Target-cell destruction attained its peak at a target-cell/ lymphocyte ratio of 1 : 100. On adding azathioprine to the culture in order to avert the cytotoxic effect, he found that the complete suppression of the cytotoxic effect required the presence of the immunosuppressive agent in the medium. MÖLLER et al. sought to counteract the cytotoxic effect of PHA- and streptolysin-stimulated lymphoid cells on fibroblast cultures by X-ray irradiation, cortisone and chloroquine. As reported by LUNDGREN et al. [1968], human granulocytes also affect human fibroblasts, as a result of which the monolayer of these cells separates from the glass surface without, however, being destroyed, as shown by their ability so be cultured afresh from the supernatant. According to LUNDGREN, heterologous antilymphocyte serum (ALS), while inducing a cytotoxic activity in lymphocytes at certain concentrations, suppresses at other concentrations the cytotoxic activity of lymphocytes induced by PHA and by other mitoblastogenic substances. TIMOTHY et al., having found the cytotoxic reaction suitable for in-vitro studies of cell-mediated immunity, recommend its quantitation. As pointed out by these authors, in order to be informative, the cytotoxic procedures require in their majority a fairly long incubation time which, however, involves a spontaneous destruction of the target cells as well as of lymphocytes. Therefore, they used the procedure described by BRUNNER: mice were sensitized with tumour cells, then, on addition of an adequate amount of splenic cells of these mice to the ^{51}Cr labelled tumour cells, they found an extensive release of Cr isotope at the end of 3 to 4 hours incubation.

Until recently the toxic effect of lymphocytes on the tissues has been generally studied on cell monolayers of firm adhesion [6] or on suspension cultures [3, 12]. Labelling with ^{14}C thymidine [6], more often with Cr isotope [3, 4, 5, 12] permits to assess the degree of cytotoxicity by the measurement of isotope release (Cr release) associated with cell destruction. It has, however, been found that in case of incomplete cellular destruction resulting from the cytotoxic effect of lymphocytes the cells still fail to release the incorporated isotope. This requires additional trypsinization of the cell cultures and measurement of the radioactivity in the "supernatant II," too. Labelled Cr is known to be released in large amounts even spontaneously after some length of time. This method thus involves various sources of error which have to be taken into account when assessing the results.

Our procedure eliminates the sources of error outlined above, the measurements as well as the calculations being simple and readily reproducible. It is based on the fact that cell cultures being submitted to regular passages fail to cling to glass surfaces if they have suffered some injury. Sensitized lymphocytes are known to aggregate within 1 to 4 hours on the cells of the culture and cytolysis ensues 24 to 48 hours later. The new feature

of this procedure consists in having the adhesion of cultured cells for its basis which makes it simpler and more sensitive than the isotope-release methods.

Autoimmune diseases give rise to the production of autocytotoxic lymphocytes against particular cells carrying autologous antigens. This imposes the task of identifying the given type of cells by the demonstration of autocytotoxic lymphocytes in the blood. With the autoimmune reaction being directed against a particular organ or cell type, then, in addition to autoantibodies, autoaggressive lymphocytes directed against it must be also demonstrable in vitro by means of lymphocytes separated from the blood. The investigation of this question must therefore centre on the citotoxity of lymphocytes to specific target cells. Growing and maintenance of specific target cell cultures is, however, far from simple. This has made us to use human amnionic cells derived from cultures of successive passages. According to BONSTEIN et al., tissue cells, while losing their specific tissue antigenicity in the course of continued passages, retain their species specific antigens. Accordingly, on the evidence of our preliminary experiments, it made no difference whether cultures of human fibroblasts or of HEP cells from human tumours or of human amnionic cell cultures were used as target cells. Without waiting for the formation of the monolayer in the glass tubes, the lymphocyte suspension to be studied was added immediately to the ^{51}Cr-labelled amnionic cells. If no cytotoxic lymphocytes were present, the amnionic cells were found to cling to the wall, to proliferate, and by the end of 20 hours 50 to 60% of the original activity was recovered from the adhering cells. If, on the other hand, there was a large number of cytotoxic cells among the lymphocytes, the amnionic cells failed to adhere, remained in suspension and were finally destroyed. The present procedure based on the adhesion of ^{51}Cr-labelled cells thus lends itself to the quantitative study of cytotoxicity. Measurement of the activity of the adhering labelled cells, and by this fact, of the suppression of their capacity of adhesion, seems to be more sensitive than recovery of the released ^{51}Cr. Though the use of amnionic cells offers no full substitute for specific target cells (thyroid, gastric parietal cells, etc.) against which the autoimmune reaction is supposed to be directed, the procedure has the advantage of being simple, reproducible and fairly informative of the autoaggressivity of lymphocytes.

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PSEUDO-SEASONAL RHYTHM OF HUMAN CEREBRAL STRESS BEARING CAPACITY IN THE PSYCHOCHRONOGRAPHIC (PCG) TEST

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Psychochronography (PCG), by means of the critical flicker-frequency series effect (CFFSE), allows to study the general stress-bearing capacity of the human brain through time perception. The pseudo-seasonal biorhythm of 504 male and 219 female subjects was determined from the decrement of the "fatigue," "adaptation," or "habituation" curve. This circannial rhythm seems to show a morbidity and mortality importance as well, and deserves attention in both therapy and prophylaxis.

The interactions between the human organism and its surroundings are not constant. Changes of the surroundings demand the adaptation of the organism, and the adaptation capacity of the organism is changing similarly. Thus, the same environmental change will exert a different stress on the individual in different points of time, owing to fluctuations in the organism's state of adaptation.

The daily (circadian) variations in the adaptation of the human organism have been extensively discussed in the literature. On the other hand, much less is known concerning the existence and importance of seasonal changes. Tromp [1, 2] found in the Netherlands that asthma was rare in winter and spring, but quite frequent in the summer, and particularly in the autumn. He observed seasonal fluctuations of the individual disease types [1] and referred to extensive data in the literature, in the background of which fluctuations of the organism's internal milieu may be hidden. Tromp proved that the composition of the blood would exhibit similar seasonal variations: the albumin level is higher in winter than in summer, the haemoglobin level has a minimum in June, the platelet count displays a peak in March and April, and the lowest figure in August, etc. Fenske and Holzmann [3] reported on the seasonal variations of dermatological diseases.

We have proved the presence of a pseudo-seasonal rhythm in 723 hospital patients by the psychochronographic test of human cerebral stress-bearing capacity, based on critical flicker-frequency-series effect (CFFSE) measurements [4].

Methodology

Psychochronography (PCG) is based on the registration of 50 critical flicker-frequency (CFF) data within a single series. Fluctuation of the CFF data of the same subject within one series under identical test conditions is called the CFF series effect (CFFSE). The subject has to watch a light source located at the end of a 40 cm long black funnel. The funnel ensures an identical distance of view, and the elimination of environmental effects. The light source is a cathode eye, controlled by the square wave signals of a generator indicating one-tenth c/s with the corresponding accuracy (in these investigations the generator was a Solartron low-frequency oscillator (Type OS.103.2.A), whereas in other cases generators designed specially for this purpose were used, and frequency output was determined by a frequency counter in each case). The light source intensity was about 1 lumen. A more precise determination of the intensity and composition of the light supplied by the indicator tube is of less significance as it is the variations of CFF data measured with the same light source which are compared.

No pre-adaptation or artificial pupil was used. The initial frequency of each series was 21.0 c/s, and the CFF data were always recorded in a descending sequence. Each measurement was evaluated.

PCG began one day after a preliminary learning registration consisting of two series which were not evaluated, and involved three daily recordings (early and late morning, and noon) for three days. Each recording included two series in an interval of 5 min. The first series contained 50 CFF data recorded continuously (continuous = c series), while the second another 50 CFF data with a 30 sec interval after every tenth series (intermittent = i series). Thus, the complete test of each subject covers 900 evaluated CFF data.

The results presented below were obtained from subjects 17 to 82 years of age, hospitalized because of different diseases. The test is performed with both eyes. Bespectacled subjects wear their glasses.

Results

CFFSE is a two-dimensional phenomenon: each CFF value is characterized by the frequency and the sequence of recording. This can be illustrated in a co-ordinate system where the horizontal axis indicates the data-recording sequence, and the vertical data reflect the frequency number. Curves consisting of such series are usually highly variable (Fig. 1).

The question may be raised whether the fluctuation of the CFFSE curves reveals a general regularity. The question can be answered on a statistical basis by processing the individual continuous (c) and intermittent (i) series separately, and selecting from each series of the two categories the maximum and minimum CFF values for groups of five data. Thus, each CFF series will be characterized by 10 maxima and 10 minima, starting with those of the first 5 CFF data, followed by the corresponding values of the second, third, etc. 5 CFF figures. Were the two point series connected, two envelope curves would be obtained, with all the other values of the series between them. If, then, the points of the envelope curves are given, a true picture can be obtained of the fluctuation of the successive data, according to the sequence of their registration. Then the selected values of all the series are averaged separately. Thus 20 averages each are rendered by the 1590 (c) and (i) series, with each average formed from the extremes of the sequentially identical 1590 data groups. Table I presents these averages for the (c) series.

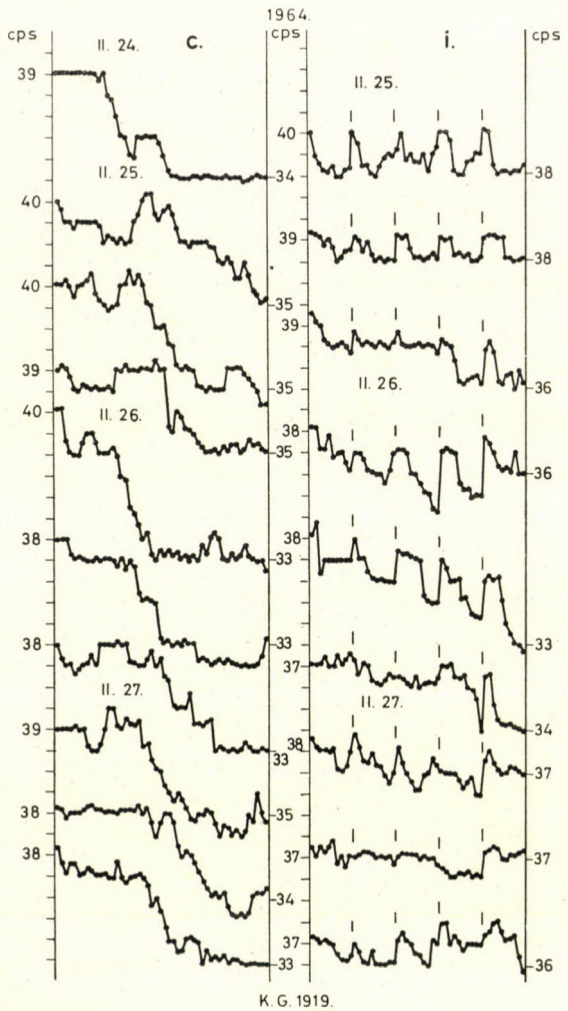

Fig. 1. K. G., 45, female, psoriatic. One training measurement recording (with only the continuous series registered), and the entire psychochronographic registration. Each curve contains 50 CFF data, from left to right, in the sequence of measurement. Left: the continuous series; right: the intermittent series, with an interruption of 30 sec after every 10 data. These interruptions are indicated. The CFF data are marked with one-tenth c/s accuracy, corresponding to the vertical axis, with a different scale for each series; the CFF data can be read off on the basis of the full c/s value next to the starting figure of the series on the left side. The c/s value next to the CFF figure closing the series is indicated on the right-side vertical axis. The days of measurement are also indicated

Fig. 2 illustrates the curves plotted on the basis of data from Table I, showing the negative exponential type considered characteristic of "adaptation," "fatigue," or "habituation." On the right, the curves of the corresponding intermittent series averages are seen where, in addition to the regenerating effect of the interruption, the negative exponential character of the curves is again manifest.

Table I

Averages of the maxima and minima per groups of 5, from a total of 1590 series consisting of 50 continuously recorded CFF data in c/s

Serial number of the group of 5	Maximum	Minimum
1	29.01	28.09
2	28.51	27.79
3	28.21	27.53
4	27.98	27.31
5	27.77	27.10
6	27.57	26.91
7	27.40	26.73
8	27.24	26.57
9	27.08	26.42
10	26.95	26.28

Table II

Monthly G averages (circannial rhythm) of 723 subjects

Month	Males		Females	
	Subjects	G (per cent)	Subjects	G (per cent)
I	39	78.58	16	77.67
II	29	80.94	14	79.11
III	44	82.37	16	80.71
IV	59	81.91	23	80.08
V	37	81.01	29	74.44
VI	56	78.46	28	75.55
VII	44	77.56	24	79.48
VIII	42	79.65	15	76.52
IX	51	80.87	17	76.71
X	41	74.72	10	72.31
XI	33	77.20	14	77.37
XII	29	82.09	13	80.42
Total	504	79.61	219	77.45

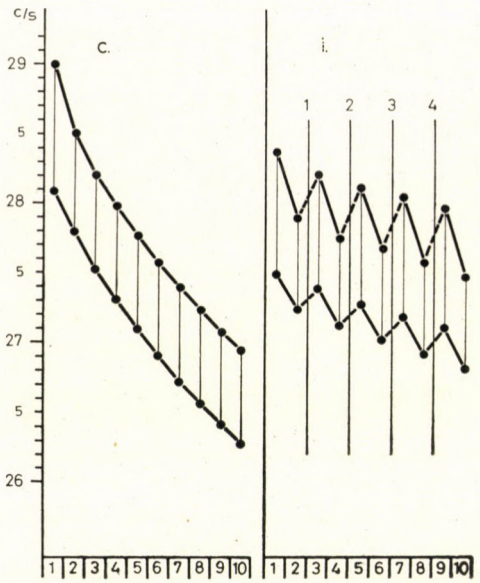

Fig. 2. Averages of maximum and minimum CFF data, by groups of 5, of 1590 CFF series pairs. Left: averages of continuously recorded series; right: averages of series recorded with four 30-sec interruptions. Vertical axis: c/s data with an accuracy of one hundredth; horizontal axis: successive groups of 5 in the sequence of recording

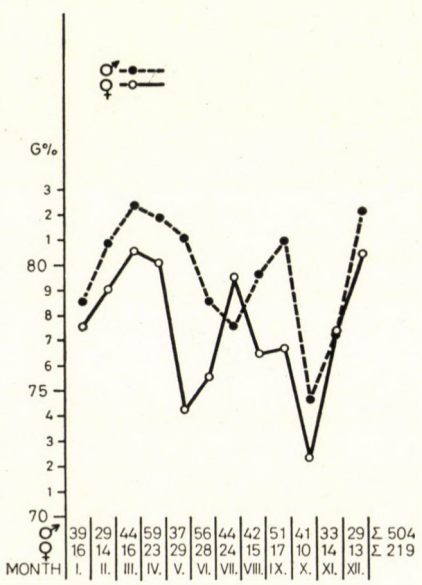

Fig. 3. Variation of monthly G-ratio average by sex. Dash line: males; full line: females. Vertical axis: G per cent; horizontal axis: months, and monthly number of subjects in the groups by sex

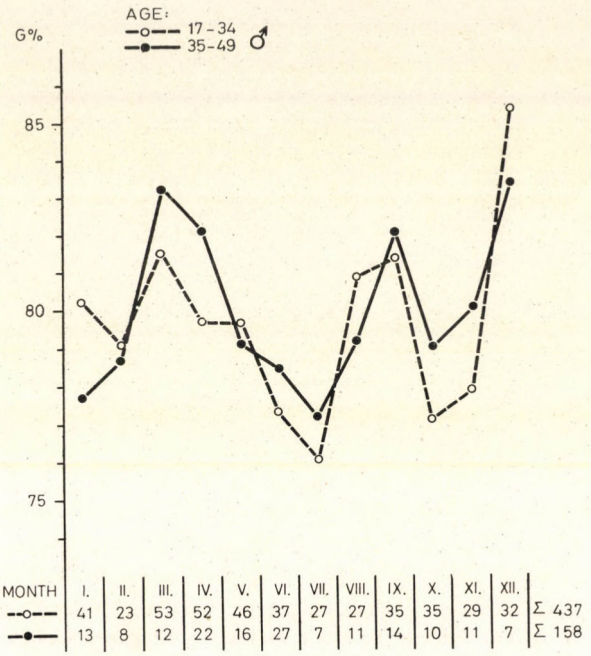

Fig. 4. Monthly G averages of two male age groups of different patients. Dash line: 17—34; full line: 35—49 years of age

	Number of subjects											
Group type	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
17—34	41	23	53	52	46	37	27	27	35	35	29	32
35—49	13	8	12	22	16	27	7	11	14	10	11	7

In the CFF series a decrease of the values occurred with regularity. The change can be characterized by a single ratio expressing the value variation range (VR) of the first 30 data in the series, i.e., the difference between the maxima and minima of the first 30 data (A), against the VR of the entire series (B). This ratio is expressed in percentage by the index $G = 100\ A/B$.

If the series changed linearly, the ratio G would amount to 60% (30 : 50 = 60 : 100). Since, however, the average decrease of the series is by a decrement, the average is characterized by $G > 60\%$.

As for the data in Table I, e.g., $A = 2.10$, $B = 2.72$ and $G = 77.30\%$.

If the G averages are broken down by months, a peculiar pseudo-seasonal fluctuation can be discovered (Table II and Fig. 3).

Fig. 3 reveals a similar but certainly not identical annual rhythm for males and females which does not correspond to the seasons.

For the sake of comparison, Fig. 4 presents the circannial rhythm of 437 young (17—34) and 158 medium age (35—49) male subjects. These two age groups are sufficiently large to compensate in the averages the differences caused by the uneven health conditions (the group of females and that of old subjects were not large enough to satisfy the above requirement and, therefore, their circannial averages cannot be accepted).

Fig. 5 illustrates the above circannial biorhythm for 158 males and 117 females, both neurotic and of mixed age.

Discussion

The ratio G shows that general feature of the CFFSE which characterizes the decrement of the average decrease of the series. This selected characteristic, usually considered typical of the "fatigue," "adaptation," or "habituation" curves [5], displays not only a circannial rhythm [6]. We have shown [7] that it is affected also by age, sex, health conditions, etc.

The facts that in a group of sufficient number of individuals the male and female subjects exhibited a similar annual rhythm, that the males in a breakdown by age revealed the same circannial rhythm, that the neurotic male and female patients displayed the same fluctuations proved that, in the case of sufficiently large groups, the pseudo-seasonal rhythm thus discovered was realistic. This is supported by DRIETOMSZKY [8] who, in his spring and autumn PCG examinations on healthy subjects over 50 years of age found the same sense of difference between the G averages for the two groups, with a high significance ($p < 0.00001$). If the group tested is homogeneous, and consists of a sufficient number of subjects, like that of the neurotic males (Fig. 5), the difference of the March and October averages will already be significant ($p < 0.05$).

This annual rhythm was observed in Budapest. Whether the same applies to other sites, requires further investigation. PCG is a psychophysical test, the essentials of which are other then the in-time distinction on which the CFF is based. CFF is the maximum frequency number, according to which the discrete character of the flickers can still be distinguished. Something entirely different is expressed by the CFFSE: it is based on the fact that, in the course of successive CFF observations, the preceding appreciations affect the subsequent results, in other words, the CFF data have an in-time sequence. This means that after the physical stimulus its trace will exert an action during the subsequent recording, the brain compares the earlier frequency observations of the series (its traces) to the actual physical frequency appreciation. In other words, the temporal distinctions are associated with differential times. These differential times are called micro-intervals since, on

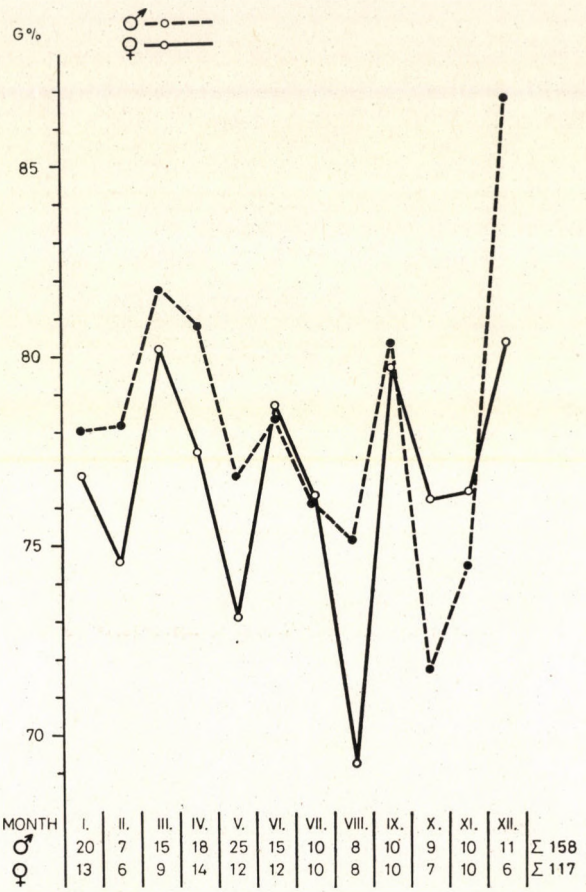

Fig. 5. **Monthly G averages of mixed age group neurotic males (dash line) and females (full line)**

Group type	Number of subjects											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Males	20	7	15	18	25	15	10	8	10	9	10	6
Females	19	6	9	14	12	12	10	8	10	7	10	6

the basis of measurement accuracy, their order of magnitude of 10^{-4} to 10^{-5} sec can readily be calculated.

The PCG records time perception, and not the time of perception. Its order of magnitude is 10^{-2} to 10^{-3} times that of the observation or reaction time, so it is related to times smaller by several orders of magnitude.

In our opinion, the CFFSE measured by PCG is of a central and not peripheral (retinal) origin. It is well-known that literature offers different

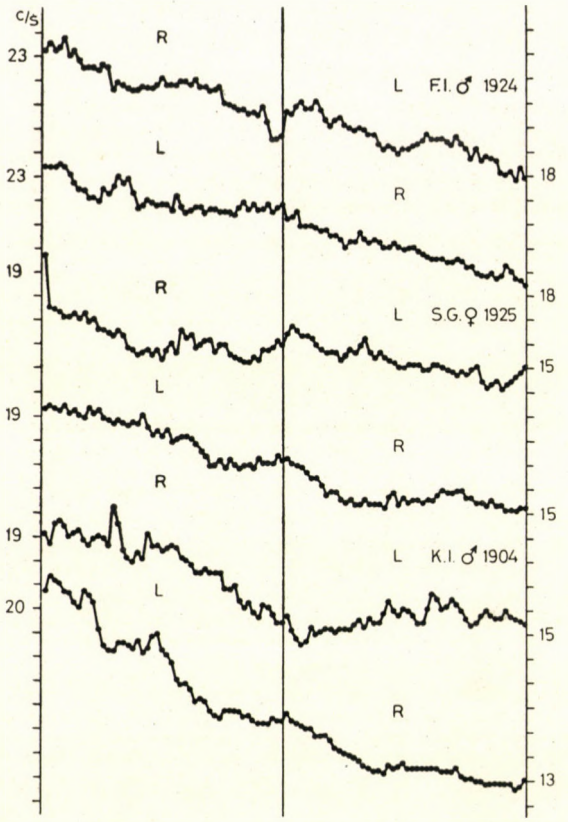

Fig. 6. Continuous series of 100 CFF data rendered by 3 subjects on the same day. The first 50 data were recorded with one, the second 50 with the other eye. R: right eye; L: left eye. Vertical axis: c/s values next to the first and last values of the series. The scale graduations indicate c/s units. No tenths are indicated. Individual CFF data of the series are shown by the points from left to right, with an accuracy of one tenth c/s, in the sequence of recording. Each point represents one measurement, not an average

standpoints on the central or peripheral generation of CFF data. As for the CFFSE, the following experiment was conducted to prove its central origin.

The same subject had to supply continuously 50 CFF data with one eye covered, then, after changing quickly the eye cover, his (her) next 50 CFF data were measured. This experiment was performed twice with each subject: once starting with his (her) right eye, and once with the left one. Had the two eyes been functioning independently with respect to the CFFSE test, the first and second series, each consisting of 50 data, would be similarly independent of each other, since the retinal variations of one eye could not influence the activity of the other retina. Fig. 6, however, reveals that the

curve plotted from the data supplied by the second eye follows that of the first eye in a regular manner, so the CFFSE is of central origin [7].

In the present paper, the circannial rhythm of the variations in cerebral stress-bearing capacity have been demonstrated by the decrease of the continuous series decrement. The CFFSE function connected to time perception exhibited a surprising annual rhythm from biometeorological aspects. This biorhythm is certainly not coincident with the seasons of the year, nor with the weather conditions prevailing in Hungary. Thus the pseudo-seasonal rhythm ought to be considered an endogenous rather than an exogenous change.

On the basis of comparative examinations carried out on healthy and diseased subjects [8] we must believe that the higher G ratio is an unfavourable symptom which refers to a reduction of the cerebral stress-bearing capacity. When comparing the G per cent of certainly healthy males and females over 50 years of age to that of three groups of the same age with frequent disease (neurosis, hypertension, cerebrovascular syndrome), the values obtained were always higher than those for the healthy subjects, with a strong significance in each case ($p < 0.01$). In investigations conducted in co-operation with Dr. S. Blazsó the same was found with children, where the patients rendered higher G-per-cent figures than the healthy subjects, with a significance of $p < 0.000001$ of the difference between healthy subjects and epileptics.

With respect to the circannial rhythm, it was remarkable that the spring peak should have essentially coincided with the spring mortality maximum observed in Budapest almost every year.

It may be of interest that Wüthrich and Rieder [9] concluded from their Swiss data collection on the seasonal incidence of sclerosis multiplex that most cases occurred in March, while the lowest number was observed in October. As for our findings, this may be thus interpreted that when the general stress-bearing capacity of the brain is at its minimum, then the frequency of sclerosis multiplex cases is at its maximum, and when PCG reflects the annual maximum of the cerebral load-bearing capacity, then the occurrence of sclerosis multiplex is lowest.

All these raise the question, as a further research target, whether it would be possible to interfere actively with this circannial rhythm and, for example, increase somehow the general stress-bearing capacity of the brain. Based on PCG testing, the results of various interventions could be measured. Furthermore, with this marked annual biorhythm recognized, when deciding on the therapy of chronic diseases, the pseudo-seasonal fluctuation should, perhaps, also be taken into consideration.

Finally, it should be mentioned that the high G-per-cent values observed at the end of the year are regarded as induced data. Around Christmas only

the most serious patients stay in hospital, everybody else would spend the season in family circle. This selection may well cause the December peak of the curve.

*

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EFFECT OF CLOFIBRATE ON WATER METABOLISM IN HYPOTHALAMIC AND ADH-RESISTANT DIABETES INSIPIDUS

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The antilipaemic drug, clofibrate, was administered orally to nine vasopressin-sensitive diabetes insipidus patients with some reserve, one patient without ADH reserve, and four ADH-resistant patients, and the resulting changes in water metabolism were examined. In the nine ADH-sensitive patients clofibrate decreased urinary output and free water clearance, and increased urinary osmolarity. In the vasopressin-sensitive patient with no ADH reserve, and in the ADH-resistant diabetes insipidus patients the drug did not affect urinary output.

The results indicated that clofibrate exerts an ADH-like effect on water metabolism, and may be useful in the treatment of vasopressin-sensitive patients with hypothalamic diabetes insipidus.

Introduction

In work on the treatment of diabetes insipidus attention has recently turned to saluretics [10, 20, 27, 38, 43], oral antidiabetics [3, 7, 15, 18, 23, 26, 29, 32, 33, 39, 40, 45, 46, 48, 49] and antiepileptics [5, 17, 44]. The advantage of these drugs is that they dispense with the need for daily ADH injections and the use of snuff powders, which can lead to rhinitis. As a result of their many side-effects, however, their prolonged administration is possible only rarely. With diuretics or chlorpropamide, hypopotassaemia and hypoglycaemia, respectively, must be reckoned with. In 1970, DE GENNES et al. [19] reported that clofibrate, which is known for its antilipaemic effect, decreases the volume of urine in diabetes insipidus. In the present investigation the effect of clofibrate on water metabolism was examined in patients suffering from ADH-sensitive hypothalamic, or ADH-resistant diabetes insipidus.

Material and methods

The diabetes insipidus patients were classified into two groups. Group one was made up of nine vasopressin-sensitive patients with a certain ADH reserve, and one patient with no ADH reserve: in every patient, the anterior pituitary functioned normally. Group two were four ADH-resistant cases. The diagnosis was established on the basis of methods detailed earlier (LÁSZLÓ et al. [29], LÁSZLÓ and CZAKÓ [28]). The ADH-reserve capacity was estimated from the change in ADH excretion during thirst or hypertonic saline infusion (CZAKÓ et al., in preparation [11]). The ineffectiveness of ADH in the second group was confirmed by the Hankiss intravenous ADH test (HANKISS [21]): water-loading combined with

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Table I

Data of the patients

No.	Patient	Age, years	Sex	Aetiology	Duration of disease, years	Pre-treatment urine volume, 24 hrs	Previous therapy	Additional information
				ADH-sensitive diabetes insipidus				
1	B. L.	58	M	idiopathic	52	10—12	Piton-snuff powder	—
2	H. L.	38	M	skull injury	4	20—25	Pitressin-tannate and Piton-snuff powder	Chlorpropamide not tolerated
3	V. K.	27	M	tuberculo-meningo-encephalitis	24	10—12	Piton-snuff powder and chlorothiazide	Could not take drugs for a long period
4	L. M.	43	M	idiopathic	1	6— 8	—	—
5	S. M.	30	F	febrile infectious disease	16	8—10	Pitressin-tannate	Piton-snuff powder and chlorpropamide not tolerated
6	B. S.	33	M	Hand—Schüller—Christian-disease	14	13—15	Piton-snuff powder, chlorothiazide	—
7	M. J.	52	M	skull injury	21	6— 8	—	—
8	R. A.	63	M	idiopathic	2	10—12	Pitressin-tannate	—
9	M. I.	67	F	idiopathic	8	12—15	Piton-snuff powder	—
10	F. P.	43	M	skull injury	19	30—40	Pitressin-tannate	Oral antidiabetics ineffective
				ADH-resistant diabetes insipidus				
11	Cs. J.	23	M	idiopathic	18	13—15	—	Diuretics not tolerated
12	T. F.	15	M	idiopathic	12	10—12	Chlorothiazide, spirono-lactone	—
13	K. I.	19	F	idiopathic	16	13—15	—	Diuretics not tolerated
14	A. I.	32	M	idiopathic	28	10—15	Chlorothiazide, spirono-lactone	—

vasopressin injection (LÁSZLÓ et al. [30]), and by the administration of long-acting pitressin tannate. None of the patients suffered from demonstrable heart or liver disease. The more important data relating to the patients are given in Table I.

The experiments were made on patients maintained on a light, mixed diet: for seven days they received no special treatment, then for 6 days they received 2.0 g of clofibrate daily.

The following parameters were determined: urinary output (l/24 hrs), urine and serum osmolarity (mosm/l), endogenous creatinine, osmolar and free water clearance (ml/min), blood sugar, cholesterol, total lipids, free fatty acids, triglycerides, serum Na^+ and K^+ (mEq/l), and Na^+ and K^+ (mEq/24 hrs) excretion. Serum and urine osmolarity was estimated by measurement of the freezing-point depression. The following methods were used: for blood sugar, an orthotoluidine procedure; for serum and urine Na^+ and K^+, flame-photometry; creatinine: BROD and SIROTA's [6] modification of the FOLIN and WU [16] method; cholesterol: the method of ZAK et al. [50]; total lipids: the vanillin-phosphoric acid method of DREVON and SCHMIT [13]; triglycerides: the method of CONNERTY et al. [9]; free fatty acids: as copper soap by ANSTALL and TRUJILLO's [2] modification of the method of DUNCOMBE [14]. The copper content of the soap was measured with diphenylcarbohydrazide according to MAHEDEVAN et al. [31].

The results were evaluated by means of Student's single-sample *t*-test.

Results

During clofibrate treatment for 6 days no substantial changes were observed in the serum cholesterol, total lipid, triglyceride and free fatty acid levels. Nor were any significant changes found in blood sugar, or in the serum and urinary Na^+ and K^+ levels.

Table II shows the results in the ADH-sensitive, hypothalamic diabetes insipidus cases. Spontaneous urinary output was considerably decreased in nine patients; this was the most pronounced in Case 2, while it was slightly modified in Case 9. Urinary osmolarity increased substantially. Serum osmolarity and endogenous creatinine clearance did not change, osmolar clearance was somewhat decreased. Free water clearance was considerably reduced in Cases 4, 5 and 7, and even became negative, thus, free water reabsorption resulted. A moderate reduction in free water clearance could be observed in the other patients.

The response of clofibrate in Case 10, who had no ADH reserve, was completely different. Antidiuresis could not be demonstrated in this patient, and the data measured remained unchanged even during clofibrate administration (because the different response of this patient from those with ADH reserve, the results have not been included in Table III).

Table III shows the results for the ADH-resistant diabetes insipidus patients. Daily urine volume was not affected significantly by clofibrate, and in Case 2 there was even a slight increase. Serum and urine osmolarity, osmolar clearance and free water clearance increased to a moderate extent, while endogenous creatinine clearance decreased slightly. In order to show how the changes developed in time, detailed data for one typical patient from each group, both before and during clofibrate therapy, are given.

Table II

ADH-sensitive

No.	Name	Age, years	Sex	Urinary output, l/24 hrs		Serum osmolarity, mosm/l	
				before treatment	after change	before treatment	after change
1	B. I.	58	M	10.9	— 6.8	299.8	+ 5.7
2	H. L.	38	M	23.3	—15.2	304.3	+ 3.6
3	V. K.	27	M	9.4	— 6.3	300.7	+ 8.6
4	L. M.	43	M	6.1	— 3.3	303.6	— 1.0
5	S. M.	30	F	11.9	— 8.3	313.1	— 3.2
6	B. S.	33	M	11.1	— 4.7	329.9	— 9.2
7	M. J.	52	M	8.3	— 5.3	316.9	—18.7
8	R. A.	63	M	12.3	— 5.5	311.8	—16.2
9	M. J.	67	F	10.5	— 1.5	316.9	+ 0.4
Mean				11.5	— 6.3	310.7	— 3.3
Standard error of the mean				± 1.61	± 1.29	± 3.25	± 3.18
					$p < 0.01$		p = NS

Figure 1 shows the data for an ADH-sensitive diabetes insipidus patient (Case 5). On the first day already, urine volume was significantly decreased by clofibrate, and on the 3rd—6th day urinary output fell to one fifth of that in the control period. No marked change could be observed in serum osmo-

Table III

ADH-resistant

No.	Name	Age, years	Sex	Urinary output, l/24 hrs		Serum osmolarity, mosm/l	
				before treatment	after change	before treatment	after change
1	C. J.	23	M	11.6	+0.4	302.4	— 1.0
2	T. F.	15	M	11.8	+2.3	291.5	+27.6
3	K. I.	19	F	12.3	—1.2	316.9	+ 2.2
4	A. I.	22	M	13.5	—1.8	317.6	— 0.1
Mean				12.3	—0.1	307.1	+ 7.1
Standard error of the mean				±0.42	±0.91	±6.27	± 6.84
					p = NS		p = NS

diabetes insipidus

Urinary osmolarity, mosm/l		Endogenous creatinine clearance, ml/min		Osmolar clearance, ml/min		Free waer clearance, ml/min	
before	after	before	after	before	after	before	after
treatment	change	treatment	change	treatment	change	treatment	change
130.9	+ 98.7	129.1	+ 9.6	3.4	−1.4	4.2	−3.2
130.7	+190.2	197.5	−49.3	7.3	−2.9	8.9	−2.0
163.6	+124.0	145.3	+31.8	3.5	−1.7	3.0	−1.2
223.9	+236.3	144.0	+18.3	3.1	−0.3	1.1	−6.2
131.1	+255.3	137.9	+15.5	3.4	−0.7	4.8	−6.1
136.8	+164.6	101.4	− 2.2	3.2	+1.5	4.5	−1.3
137.7	+198.8	125.4	+25.7	2.5	−0.2	3.2	−4.4
195.6	+ 25.4	184.7	− 4.2	5.4	−2.0	3.1	−1.8
180.5	+ 44.6	175.0	−45.0	4.2	−0.1	3.1	−1.0
158.9	+148.7	148.9	+ 0.02	4.0	−0.9	4.0	−3.0
±11.40	± 27.05	±10.32	±82.56	±0.49	±0.43	±0.71	±0.69
	$p < 0.001$		p = NS		p = NS		$p < 0.01$

NS = not significant

larity or in endogenous creatinine clearance. In contrast, urine osmolarity increased considerably during the treatment, and osmolar clearance was slightly altered. On the other hand, there was a pronounced decrease in free water clearance, which became negative from the third day of treatment.

diabetes insipidus

Urinary osmolarity, mosm/l		Endogenous creatinine clearance, ml/min		Osmolar clearance, ml/min		Free-water clearance, ml/min	
before	after	before	afrer	before	after	before	after
treatment	change	treatment	change	treatment	change	treatment	change
161.0	−29.0	79.5	+12.6	4.3	−0.7	3.8	+0.9
114.3	+23.3	68.8	− 7.9	3.2	+0.9	5.0	+0.7
86.8	+14.2	138.3	+ 4.8	2.3	+0.6	6.2	+0.1
142.7	+ 8.4	189.1	−72.0	4.2	+0.1	5.2	−1.3
126.2	+ 4.2	118.9	−15.6	3.5	+0.2	5.0	+0.1
±16.27	±11.49	±24.78	±19.26	±0.41	±0.34	±0.50	±0.49
	p = NS		p = NS		p = NS		p = NS

NS = not significant

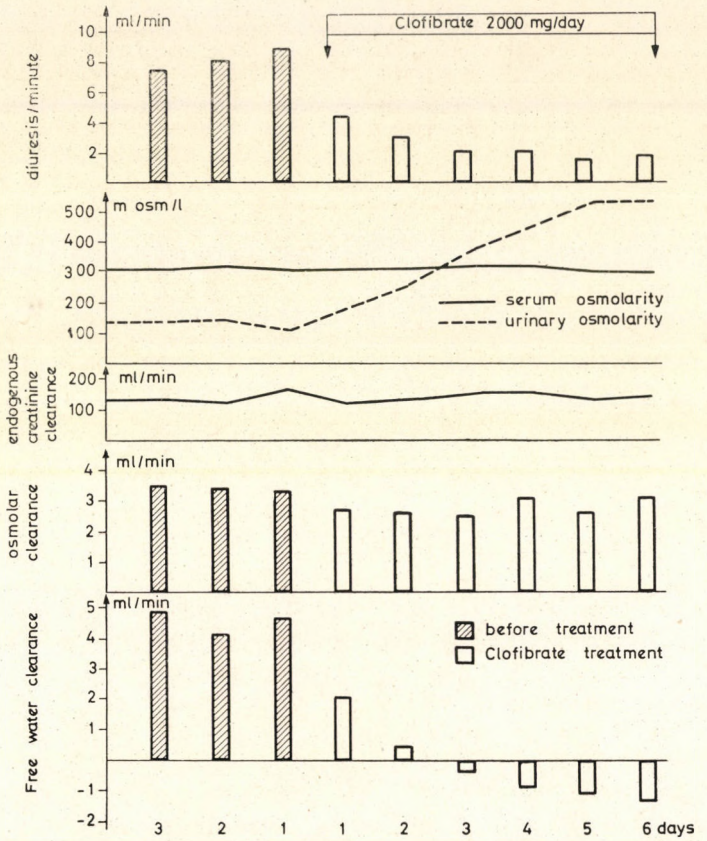

Fig. 1

Figure 2 shows the effect of treatment in Case 1, an ADH-resistant diabetes insipidus patient. It can be seen that the daily urine volume did not change substantially. Urinary osmolarity remained at a low level during the administration of clofibrate. There was no significant change in serum osmolarity, endogenous creatinine clearance or osmolar clearance, nor could any difference be detected in free water clearance.

Discussion

The effect of clofibrate on water metabolism has been investigated. There was a decrease in daily urinary output in nine ADH-sensitive patients. The drug did not affect the extensive polyuria in a tenth ADH-sensitive patient without ADH reserve. Similarly, there was no decrease in four ADH-

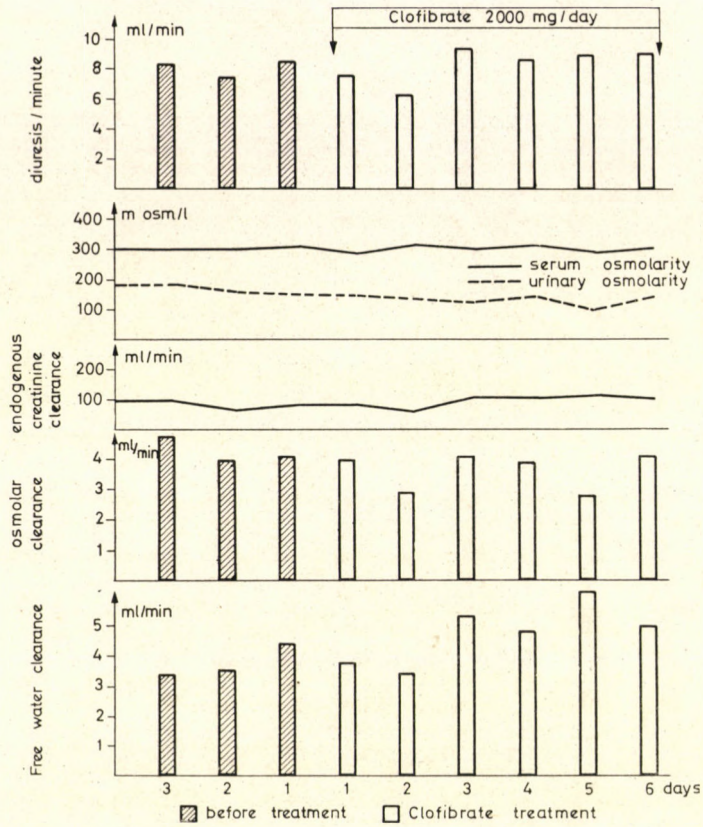

Fig. 2

resistant diabetes insipidus cases. There seem to be few reports on the diuretic action of clofibrate: in 1970 DE GENNES et al. [19] reported that when the chemically identical preparation Atromide-S or Lipaflavone was administered in daily doses of 2—2.5 g, in three diabetes insipidus patients daily urinary output decreased to about one third of the initial. In normal patients the diuresis elicited by hyperhydration was affected by the drug to a small extent. BERTRAND [4], UHLICH [47] and DECOURT [12] reported effective clofibrate treatment in patients with hypothalamic diabetes insipidus.

The antilipaemic effect of clofibrate has been known for about ten years. From among the individual lipid constituents it exerts the greatest effect on the serum triglycerides (CARLSON et al. [8], OLIVER [34, 35]). The change of the cholesterol level is not clear-cut (HELLMANN et al. [22], HOOD et al. [24, 25], ROBINSON [41]). It is certain that in the case of normolipidaemia clofibrate does not induce important alterations in the blood lipids (OLIVER [36]).

The mechanism of action of clofibrate on water metabolism is unknown. DE GENNES et al. [19] mention that the drug had an effect similar to that of chlorpropamide. Our own results allow some suggestions. A renal site of action independent of the ADH mechanism cannot be accepted since antidiuresis does not result in ADH-resistant diabetes insipidus. The drug does not affect solute excretion but decreases free water clearance. This suggests an ADH-like effect, and this assumption is also supported by the observation that clofibrate did not produce a significant difference in water metabolism in vasopressin-resistant diabetes insipidus patients. However, the renal effect of the drug is not exactly the same as that of ADH, for the polyuria remained unchanged in the ADH-sensitive diabetes insipidus patient without ADH reserve. Thus ADH must be present for clofibrate to affect water metabolism, the effect being exerted either by a mobilization of the ADH reserve or by the potentiation of the action of the small amount of vasopressin reserve. Finally, clofibrate may reduce water excretion by a change in the metabolism of ADH.

In the present stage of our experiments we are unable to take a stand in preference of one or other of the possibilities. In order to elucidate the mechanism of the effect we are now carrying out quantitative ADH reserve determinations in our diabetes insipidus patients, and we also wish to study whether there is a relation between the degree of clofibrate antidiuresis and the extent of the mobilizable ADH reserve. In addition, we are seeking an answer to the question of whether ADH excretion increased during the treatment.

No noteworthy side-effect was observed during our examinations. Five of the patients have now been taking the drug for six months, with minor interruptions, without any serious complains. Our findings with prolonged treatment will be reported in detail in a future paper.

Our results indicate that clofibrate is an effective drug in the therapy of diabetes insipidus. Its advantage is that in can be administered orally, without hypopotassaemic or hypoglycaemic side-effect, and that it can also be used when diabetes insipidus is accompanied by a failure of the anterior pituitary.

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ÜBER PATHOLOGISCHE VERÄNDERUNGEN DER LUNGE BEI DER INTERMITTIERENDEN HYPERBAREN SAUERSTOFFTHERAPIE*

Von

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Einführung

Bald nach der Entdeckung des Sauerstoffs und Erkenntnis seiner fundamentalen Bedeutung für animalische Prozesse wurde auf die toxische Wirkung dieses »Lebensgases« hingewiesen. Der Entdecker PRIESTLEY selbst hat eine »mögliche schädigende Wirkung bei Überdosis« vermutet. Der Begriff der Sauerstoffvergiftung wurde von BERT 1878 geprägt. In den folgenden knapp 100 Jahren sind viele Untersuchungen publiziert worden. Im wesentlichen handelt es sich um Untersuchungen über Veränderungen des Zentralnervensystems und der Lunge (Lit. bei BEAN, JACOBSON, WALTHER). So hat man besonders hinsichtlich der Schädigung des zentralen Nervensystems den Begriff des Lorrain–Smith-Effektes [16, 17] herausgearbeitet, wonach der Angelpunkt der Sauerstoffvergiftung die Störung der Gasaustauschfunktion der Lunge darstellt. Auch die übrigen pathologischen Befunde wurden auf diese gestörte Lungenfunktion zurückgeführt.

Bei diesen Veränderungen handelt es sich jedoch um organspezifische Endzustände der Sauerstoffvergiftung. Aufgrund eigener enzymhistochemischer Studien an Herz, Leber, Niere, Hoden und Milz sowie in Verbindung mit den biochemischen Untersuchungen — erinnert sei hier an die Arbeiten von HAUGAARD, GILBERT, DICKENS, LAMBERTSEN, WOOD u. v. a. (Lit. bei HAUGAARD, WALTHER) — handelt es sich vielmehr um eine allgemeine Vergiftung mit Störung des Zellmetabolismus. Als primäre Reaktionen des hyperbaren Sauerstoffs sind die Oxydation, Peroxydation und Radikalbildung anzuführen. Sekundär kommt es dann zu Enzymblockierungen, Schädigungen von Proteinen, Lipiden und damit zu mannigfachen Störungen des Zellmetabolismus. Die tertiären pathologisch-histologischen Veränderungen werden dann an den verschiedenen Organen in Form von Zellnekrosen (z. B. am

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Gehirn), Membranverdickungen mit entzündlichen Veränderungen (z. B. in der Lunge) oder einer Nekrobiose von Herzmuskelfasern nachweisbar (siehe Abb. 1).

Reaktionskette des hyperbaren Sauerstoffs

Primär Oxygen	Oxydation, Peroxydation, Radikale
	↓
Sekundär (Zell-Metabolismus)	Proteine, Enzyme, Lipide
	↓
Tertiär (Histologischer Effekt)	Organspezifische Veränderung

Abb. 1. Reaktionsverlauf der Sauerstoffvergiftung. Die Abbildung zeigt die primären Reaktionen des Sauerstoffs, die sekundär zur Störung des Zellmetabolismus führen und im Spätstadium die bekannten histologischen Veränderungen bewirken

Durch eine Behandlung mit Sauerstoff im Überdruck ist es in erster Linie aufgrund des Henryschen Gasgesetzes möglich, das Sauerstoffangebot in der Peripherie zu erhöhen. Diese Therapie hat daher breiten Eingang in fast alle klinische Bereiche gefunden. Die mannigfachen Indikationsstellungen mußten jedoch alsbald einer nüchternen Betrachtung weichen. Dennoch sind noch genügend Fälle vorhanden, bei denen nur eine hyperbare Oxygenation therapeutische Erfolge bringt.

Da eine längere kontinuierliche hyperbare Oxygenation aufgrund der toxischen Wirkung des Sauerstoffs nicht möglich ist, wird in der Praxis die intermittierende Therapie angewandt. In der Regel erfolgt im Abstand von 6—8 Std. eine zweistündige Hyperoxie. Die Frage bestand nun, ob diese Therapie außerhalb des toxischen Bereiches liegt.

Material und Methode

Es wurde daher eine Tierversuchsserie an Ratten über 2 volle Tage durchgeführt, in der im Abstand von 6 Std. — was dem Tagesrhythmus entspricht — eine zweistündige Hyperoxie bei 3,8 Ata erfolgte. Da der klinische Befund der Tiere nach der 3. Hyperoxie und Beginn der 4. sehr schlecht war und es zu befürchten war, daß die Tiere die 4. Hyperoxie nicht überstehen würden, wurde der Druck hier auf 2,8 Ata gemindert. (Die Beschreibung der genauen Versuchsanordnung siehe bei v. LIEVEN et al.) Nunmehr soll über weiterhin durchgeführte histologische Untersuchungen der Lunge und eine Bestimmung der osmotischen Resistenz der Erythrozyten berichtet werden.

Ergebnisse

Die histologische Untersuchung der Lunge zeigt eine ausgedehnte Entzündung, die sich ab der 3. und 4. hyperbaren Oxygenation entwickelte. Daneben waren auch die typischen Veränderungen der Verbreiterung der Alveolarsepten, der Ödemablagerung in den einzelnen histologischen Strukturen der Lunge ausgebildet. Eine Bestimmung der osmotischen Resistenz der

Erythrozyten ließ keine nachweisbare Veränderung erkennen. Dies ist sowohl hinsichtlich des jeweiligen Vergleichs zwischen den Kontrollgruppen zu den einzelnen Zeitpunkten als auch hinsichtlich des Mittelwertes über die gesamte Zeit zu sagen.

Diskussion

Bisher publizierte Untersuchungen zeigten, daß sich bei den Tieren eine metabolische Acidose entwickelte. Enzymbestimmungen im Serum ließen einen deutlichen Anstieg der LDH und SGOT erkennen. Weiterhin war in fast allen untersuchten Parametern ein deutlicher Erholungseffekt nach oder teilweise schon während der 4. hyperbaren Oxygenation, die nur mit einem Druck von 2,8 Ata erfolgte, festzustellen [10, 11].

Die pathologisch-histologischen Befunde an der Lunge sind angesichts der Ausbildung einer metabolischen — nicht respiratorischen — Acidose von besonderer Bedeutung: Trotz schwerer pathologischer Veränderungen an der Lunge, die sicher eine Störung der Gasaustauschfunktion bedeuten, kam es nicht zur Hyperkapnie des Blutes. Daß im Gewebe eine Kohlendioxyd-Retention besteht, ist bekannt [4, 12], jedoch ist diese ursächlich nicht mit der vorhandenen Lungenschädigung in Beziehung zu bringen. Die hier genannten Befunde weisen wieder einmal darauf hin, daß bei der hyperbaren Oxygenation nicht die Lunge das primär geschädigte Organ ist.

Die Bestimmung der osmotischen Resistenz der Erythrozyten wurde durchgeführt, da unter der hyperbaren Oxygenation eine Membranschädigung nachgewiesen ist [3, 6, 7, 8, 9, 13, 15, 19]. Eine solche konnte im Verlauf der intermittierenden Therapie nicht beobachtet werden. Dieser Befund ist für die Interpretation der Veränderungen der Serumenzyme (LDH, SGOT) von Bedeutung, da hieraus zu schließen ist, daß die Enzymaktivitätserhöhungen [11] nicht das Resultat einer Hämolyse darstellen können. Es muß sich hier demnach um Gewebsenzyme handeln, deren Austritt mit der allgemeinen Membranschädigung der Zellen sowie deren Organellen in Beziehung zu setzen ist.

Zusammenfassung

Anhand eigener enzymhistochemischer, histologischer und blutchemischer Untersuchungen sowie den Angaben in der Literatur stellt sich die hyperbare Sauerstoffvergiftung im Gegensatz zum Lorrain–Smith-Effekt als eine allgemeine Vergiftung dar, die aber die morphologischen und funktionellen Systeme des Individuums mit unterschiedlicher Intensität schädigt.

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

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ANNOUNCEMENT OF AN IAEA SYMPOSIUM

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Date: 15—19 July 1974

Location: Knoxville, Tennessee, USA

Organizers: International Atomic Energy Agency
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Medical Applications Section

This Symposium, a sequel to the symposium on the same subject held by the International Atomic Energy Agency in Rotterdam in 1970, will be concerned with all those applications of radionuclides in clinical medicine and research that involve investigation of the temporal patterns of uptake, metabolism, clearance or excretion of administered radioactive materials.

Emphasis will be placed on the methods used and their potentialities. Topics covered will include technique (radioactivity measurement, data analysis, etc.), applications in studies of the function of organs and systems of the body (specific organs, the circulatory system, etc.) and applications in studies of the metabolism of substances in the body (minerals, proteins, radiopharmaceuticals etc.). Studies based on scientigraphic techniques will be excluded except insofar as they are concerned with dynamic situations.

Further information and forms to accompany abstracts of papers intended for presentation at the Symposium may be obtained from national authorities for atomic energy matters. Abstracts must be submitted through these authorities so as to reach the International Atomic Energy Agency by 8 February 1974.

PRESS RELEASE

INTERNATIONAL SYMPOSIUM ON WOUND HEALING

8—12 April, 1974, Rotterdam, the Netherlands

At the new premises of the Medical Faculty of the Erasmus University, Rotterdam, an International Symposium on Wound Healing will be held from 8—12 April, 1974.

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Biological, biomechanical, physiological, pharmacological, pathological and clinical aspects of the process of healing in different tissues will be discussed. These topics will be dealt with by authorities of international repute.

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Experts from all over the world are invited to attend the Symposium, to which a book exhibition, a scientific exhibition as well as a technical exhibition of medical equipment and pharmaceutical products will be attached. Those, who intend to present a paper should submit an abstract of maximum 300 words before 15 December, 1973, to the Secretariat.

For further information please apply to the Secretariat, c/o Holland Organizing Centre, 16 Lange Voorhout, The Hague, the Netherlands.

The Hague, 8th August, 1973

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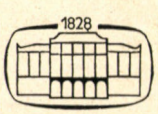

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РЕЗЮМЕ

ИЗМЕНЕНИЯ ПРИ ИСТИННОЙ ПОЛИЦИТЕМИИ, ВЫЯВЛЯЕМЫЕ ПРИ ПОМОЩИ РЕНТГЕНОВСКОГО ИССЛЕДОВАНИЯ ГРУДНОЙ КЛЕТКИ

Я. ДЬЯРМАТИ, Д. НАДЬ и Я. СЕГЕДИ

Авторы анализировали данные обыкновенного рентгеновского исследования грудной клетки 49 больных истинной полицитемией, и они в 35 случаях обнаружили измененные условия гемодинамики, т. е. кардиопульмональные рентгенологические изменения, объясняемые с основной болезнью. Частота изменений показала связь со стадиями болезни, и они были, за исключением фиброза легких, как правило обратимыми. Рентгенологические симптомы, наблюдаемые в фазе обострения болезни, уменьшались в фазе ремиссии, и в отдельных случаях они совершенно развивались обратно.

ОПЫТ РЕНТГЕНОВСКОГО ОБЛУЧЕНИЯ ЗОБНОЙ ЖЕЛЕЗИ ПРИ ЛЕЧЕНИИ ХРОНИЧЕСКОГО ПРОГРЕССИРУЮЩЕГО ПОЛИАРТРИТА

Л. САНТО, Й. ФЮЛЕП, М. ФЕХЕР и Ф. ГЁРГЕНЬИ

На основе роли зобной железы в различных аутоиммунных процессах авторы проводили в связи с лечением хронического прогрессирующего полиартрита рентгеновское облучение зобной железы. Они сообщают данные лечения 51 больного, и на основе хороших результатов рекомендуют этот вид лечения, как вспомогательную терапию хронического прогрессирующего полиартрита. В почти двух третьях случаев им удалось выявить терапевтический эффект рентгеновского облучения не только непосредственно после проведения вмешательства, но и по истечении шести месяцев и двух лет. Подчеркивается, что у пожилых больных наблюдались хорошие результаты, тогда как при ювенильном хроническом прогрессируюшем полиартрите были получены гораздо более скромные терапевтические результаты.

ИЗУЧЕНИЕ ЛАБОРАТОРНЫХ МЕТОДОВ, СЛУЖАЩИХ ВЫЯВЛЕНИЮ ДИФФУЗНОГО ВНУТРИСОСУДИСТОГО СВЕРТЫВАНИЯ (ДВС) НА ОСНОВЕ МОДЕЛЬНОГО ЭКСПЕРИМЕНТА

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В целях изучения пригодности тестов, применяемых для диагноза ДВС, авторы вызывали у кроликов разовой инъекцией эндотоксина свертывание или расстройство свертывания. Они изучали изменения факторов свертывания плазмы и проводили также исследование теста этанолового желатинирования. Содержание продуктов деградации фибриногена (ПДФ) в плазме и сыворотке определяли методом иммунноэлектрофореза.

В препаратах больше всего информаций предоставляло определение числа тромбоцитов, факторов II и X, однако разница между патологическими величинами была индивидуально очень различной. После вмешательства в сыворотке почти во всех случаях был выявлен ПДФ. Тестом этанолового желатинирования в половине подопытных животных был получен положительный результат. Гистологической техникой в микроциркуляционной системе печени и почки животных не удалось выявить фибрина.

Результаты опытов на животных подкрепляют клинические усилия, направленные на то, что при предположении наличия ФПД в целях постановки правильного диагноза и выбора терапии необходимо провести по возможности больше простых и информативных исследований.

ОПРЕДЕЛЕНИЕ СОДЕРЖАНИЯ ГИДРОКСИПРОЛИНА В МОЧЕ БЕРЕМЕННЫХ

И. ПИУКОВИЧ и Й. МОРВАИ

Авторами было изучено выделение гидроксипролина в моче 10 небеременных и 102 беременных женщин. Повышенное выделение гидроксипролина наблюдали только во второй половине беременности. В случаях угрожающего выкидыша и преждевременных родов только в последней группе удалось выявить достоверно меньшее выделение, чем при нормальной беременности.

ИЗУЧЕНИЕ КЛЕТОЧНОГО ИММУНИТЕТА ПРИ ПОМОЩИ ГОМОЛОГИЧНОГО АНТИГЕНА ПРИ ITP

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В культурах лимфоцитов 12 больных ITP и 10 здоровых лиц была изучена лимфобластозная трансформация как клеточная иммунная реакция на специфические (тромбоцитарные) и неспецифические (PHA) антигены. Тромбоцитарные антигены вызывают у большей части больных ITP бластозную трансформацию, тогда как в культурах лимфоцитов здоровых лиц этого явления не наблюдается. Результаты указывают на то, что в патомеханизме ITP играет роль клеточный иммунитет. Описанный метод повидимому пригоден также для диагностики этого заболевания.

ОБМЕН СТЕРОИДНЫХ ГОРМОНОВ У ЖЕНЩИН, СТРАДАЮЩИХ ГИРСУТИЗМОМ, ПОСЛЕ ЛЕЧЕНИЯ STC 407 И ЦИПРОТЕРОНАЦЕТАТОМ

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1. Под влиянием лечения препаратом STC 407 уменьшается количество прекурсоров тестостерона, в значительной мере влияющих на уровень тестостерона в плазме. На это указывает достоверное уменьшение количества кетостероидных фракций, выделяемых мочой.

2. Изменяется соотношение уровня DEA-S и A-S в плазме: ввиду того, что A-S является продуктом распада DEA-S, изменение указывает на то, что кроме предполагаемой до сих пор гипоталамической точки приложения действия, возможно, что STC 407 оказывает также непосредственное действие на ферменты.

3. Ципротеронацетат не вызывает достоверного изменения выделения большинства фракций кетостероидов. Согласно этому наблюдению клиническая эффективность препарата основывается на соперничестве, развертываемом на уровне внутриклеточных рецепторов.

АНОМАЛЬНАЯ ПОЛОСА ИЗОЭНЗИМА ЛАКТАТДЕГИДРОГЕНАЗЫ (ЛДГ) ПРИ ЭПИДЕМИЧЕСКОМ ГЕПАТИТЕ

И. ЛИТТЕР, К. ЙОБСТ и К. БАРНА

В связи с изучением изоэнзима ЛДГ системной сыворотки больных острым гепатитом у 25-летней больной в период от 3—16-го дня болезни между фракциями $ЛДГ_2$ и $ЛДГ_3$ изоэнзима появилась аномальная, так называемая HP-полоса, выявляемая также при повторных исследованиях. После излечения болезни картина изоэнзима стала нормальной и полоса HP также исчезла.

КРАСНЫЙ ГЛАЗ ПОЧЕЧНЫХ БОЛЬНЫХ

Л. ГОФМАН, Т. ШЮЛЕ и И. АГОШТОН

Авторы наблюдали у четырех больных хроническим заболеванием почек явление «красного глаза», вызванное отложением кальция. В одном наблюдении установили гиперкальциемию и нормофосфатемию, в двух случаях гипокальциемию и нормофосфатемию и у одного больного нормокальциемию и гиперфосфатемию. В последнем случае явление «красного глаза» развилось во время хронического гемодиализа. Согласно данным гистологического исследования коньюнктивы в базальных клетках эпителиального слоя отложился кальций. В двух наблюдавшихся случаях отложения кальция были обнаружены также в тучных клетках коньюнктивы. Явление можно привести в связь с мастокальцифилаксией.

На основе вышеописанных наблюдений и литературных данных авторы устанавливают, что с точки зрения патогенетики существуют несколько типов «красного глаза».

ГЕМОДИНАМИЧЕСКИЕ ОСНОВЫ ПРОТИВОАНГИНОЗНОГО ДЕЙСТВИЯ РАЗДРАЖЕНИЯ НЕРВА ПАЗУХИ СОННОЙ АРТЕРИИ

Ф. ШОЛЬТИ, З. САБО, Ф. РЕНЬИ-ВАМОШ, К. ШАРАИ и Л. ФЕДИНА

Авторами было изучено действие раздражения нерва каротийной пазухи на кровоток важнейших органов (сердца, мозга, почек, мышцы и кожи) и на регулирование кровообращения.

Анализ данных кровообращения был проведен у 16 собак во время раздражения пазухи сонной артерии, причем соответствующие параметры 40 собак служили контролями.

Для раздражения нерва каротидной пазухи авторы использовали радиочастотный стимулятор пазухи сонной артерии Медтроник, со следующими параметрами: напряжение = 3—6 в, частота раздражений = 30—50 гц, продолжительность импульса = 350 μсек. Минутный объем и скорость кровообращения авторы определяли методом дилюции красителя после введения ивенсовой синьки, а кровоток отдельных органов — методом одновременного введения меченого ^{86}Rb и ^{131}J антипирина. Под влиянием раздражения достоверное понижение показали минутный объем, сосудистое сопротивление в большом кругу кровообращения и скорость кровотока; исчисленная работа давления левого желудочка уменьшалась в среднем на 40%. В венечных артериях, вопреки понижению кровяного давления и минутного объема, наблюдалось ускорение кровотока, в то время как в мозге, почках, коже и мышце кровоток под влиянием раздражения нерва пазухи сонной артерии показал некоторое замедление. Доля минутного объема сердца показала под влиянием раздражения пазухи сонной артерии значительное увеличение.

АНАЛИЗ ДАННЫХ ЭКГ- И ВЕКТОРОКАРДИОГРАФИЧЕСКИХ ИССЛЕДОВАНИЙ ПРИ СЛУЧАЙНОЙ И ЭКСПЕРИМЕНТАЛЬНОЙ ГИПОТЕРМИИ

И. ПРЕДА и П. КЕНЕДИ

В связи с 5 наблюдениями случайной гипотермии авторы анализируют характерные изменения кривых ЭКГ, вызванные гипотермией, с особым вниманием на то, что между гипотермическими изменениями ЭКГ и мерцанием желудочков, предположительно, существует определенная связь. В дальнейшем они в экспериментах на собаках успешно воспроизводили вызванные гипотермией изменения кривых ЭКГ и ВКГ. Авторы стремились определить точную локализацию волны J при помощи эпикардиальных отведений и их производных. Они установили, что волна J относится отчасти к деполяризации и отчасти к реполяризации. Ее причиной является крайнее запаздывание активации постеробазальной области.

ЭСТРОГЕНЫ В МОЧЕ ЗДОРОВЫХ ЕГИПТЯН И БОЛЬНЫХ БИЛЬГАРЦИОЗНОЙ ГЕПАТОСПЛЕНОМЕГАЛИЕЙ

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Авторы определяли общие величины эстрогенов и фракций эстрона, эстрадиола и эстриола у здоровых египтян и у больных гепатоспленомегалией.

Больные были распределены на три группы: взрослые мужчины, взрослые женщины и женщины постклимактерического периода.

У мужчин и у женщин постклимактерического периода наблюдается тенденция к повышению концентрации эстрогенов. Повышение фракции эстрадиола более достоверное; это наиболее активная фракция.

У молодых взрослых женщин общие величины эстрогенов и фракций эстриола оказались достоверно пониженными.

В материале биопсии и функциональных проб печени не удалось выявить корреляции между женскими клиническими признаками и уровнем эстрогенов.

Обсуждаются результаты и выдвигаются некоторые положения об ответственности гипопитуитаризма и target-органа.

ОБРАЗОВАНИЕ ВОДОРАСТВОРИМОГО ДЕГИДРОЭПИАНДРОСТЕРОНСУЛЬФАТА И АНДРОСТ-5-ЕН-3β, 17β-ДИОЛ-3-СУЛЬФАТА В КОЖЕ ЧЕЛОВЕКА IN VITRO

И. ФАРЕДИН, И. ТОТ и М. ЮЛЕС

Срезы кожи живота здоровых женщин и мужчин были инкубированы [4-^{14}C]дегидроэпиандростероном в условиях in vitro. Из полученных многочисленных радиоактивных метаболитов дегидроэпиандростеронсульфат, андрост-5-ен-3β, 17β-диол и андрост-5-ен-3β,17β-диол-3-сульфат были изолированы и идентифицированы методом обратной изотопной дилюции. Было подтверждено, что как женская, так и мужская кожа живота обладает Δ^5-3β-гидростероидосульфокиназной аквитностью, катализирующей сульфоконьюгацию не только дегидроэпиандростеронового субстрата, но и андрост-5-ен-3β,17β-диола, образующегося во время пятичасовой инкубации. Обсуждается значение водорастворимых стероидных сульфатов, образующихся в коже.

ДИАГНОСТИЧЕСКОЕ ЗНАЧЕНИЕ РЕВМАТОИДНОЙ РОЗЕТКИ

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Авторами была изучена частота положительности ревматоидной розетки у 57 больных ревматоидным артритом, 39 больных с другими аутоиммунными заболеваниями и у 53 контрольных больных. Было установлено, что ревматоидная розетка была положительна в половине случаев ревматоидного артрита, в одной пятой части случаев других аутоиммунных заболеваний и у двух из 53 больных другими заболеваниями. 2. Между числом ревматоидной розетки и сывороточной концентрацией фактора ревматизма нет прямой связи. 3. Положительность ревматоидной розетки до известной степени параллельна активности симптомов. Ввиду того, что кроме фактора ревматизма ревматоидная розетка также связана с анти-IgG-действием лимфоидных клеток, отчасти потому, что на поверхности клетки, образующей розетку, возможно, присутствует фактор ревматизма, отчасти потому, что она непосредственно целлулярно чувствительна к иммуноглобулину, две реакции дополняют друг друга, хотя их клиническая ценность и в дальнейшем остается сомнительной.

ДЕЙСТВИЕ ГЕПАРИНА НА КОНЦЕНТРАЦИЮ ИНСУЛИНА В СЫВОРОТКЕ И В ПЛАЗМЕ

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Хейлс и Рендл выявили радиоимунным методом, применяющим два противотела, в гепариновой плазме и в гепаринизированной сыворотке меньшую концентрацию инсулина, чем в нативной сыворотке. При определении радиоимунным методом *Циглера* и сотрудников, применяющим только одно противотело, гепарин не оказал влияния на величину инсулина.

Действие гепарина связано с наличием второго — противогаммаглобулинового — противотела. Артерициально высокая концентрация инсулина в сыворотке возникает в результате действия комплемента, тормозящего реакцию второго антитела, и перекрестной реакции между вторым противотелом и человеческим гаммаглобулином. Ввиду благоприятного действия гепарина на антикомплемент и на зону оптимальной преципитации рекомендуется определять инсулин радиоимунным методом с двумя противотелами в гепариновой плазме, а не в сыворотке. Концентрация гепарина в 1,0 МЕ/мл крови достаточна для обеспечения благоприятного эффекта гепарина.

ЦИТОТОКСИЧЕСКОЕ ДЕЙСТВИЕ ЛИМФОЦИТОВ ЧЕЛОВЕЧЕСКОЙ КРОВИ В ГОМОЛОГИЧНЫХ ТКАНЕВЫХ КУЛЬТУРАХ

Г. САБО, Г. ГАСТОНЬИ, Д. СЕГЕДИ, Б. ФЕКЕТЕ и Д. ПЕТРАНЬИ

Сообщается новая радиоизотопная техника мечения ^{51}Cr для определения цитотоксического действия человеческих лимфоцитов. Сущность метода заключается в том, что из культуры меченых амнионовых клеток на стекле можно создать так наз. «monolayer», однако, прилипанию препятствуют цитоагрессивные лимфоциты, причем разница радиоактивности становится легко определимой. Метод быстрый, результаты можно оценить в пределах сутки и они хорошо воспроизводимы.

Авторы изучали этим методом цитотоксическое действие лимфоцитов больных, страдающих аутоиммунными заболеваниями, и у контрольных лиц. Они установили, что лимфоциты больных, находящихся в активной фазе аутоиммунного заболевания и не получивших лечение подавлением иммунитета, оказывают значительное токсическое действие на амнионовые клетки человека. В фазе ремиссии, наступающей без лечения, цитотоксичность лимфоцитов значительно меньше.

Под влиянием иммуносупрессивного лечения деструкция клеток лимфоцитами в существенной мере снижается.

Описанный метод можно использовать для оценки степени целлюлярной (cell-mediated) иммуноагрессии и агрессии и эффективности медикаментозного подавления иммунологической системы, как в случае аутоиммунных, так и гетероиммунных (напр. трансплантационных) реакций. В остром эксперименте через час после принятия разовой дозы 100 мг 6-меркаптопурина прекратилось выраженное цитотоксическое действие лимфоцитов взрослого больного ревматоидным артритом на культуру человеческих амнионовых клеток, и оно даже по истечении трех часов еще было значительно меньшим, чем до принятия лекарства.

ЛОЖНО-СЕЗОНАЛЬНЫЙ РИТМ СПОСОБНОСТИ К ПЕРЕНЕСЕНИЮ ЦЕРЕБРАЛЬНОГО СТРЕССА В ПСИХОХРОНОГРАФИЧЕСКОМ ТЕСТЕ (ПХГ)

К. АКОШ и А. М. АКОШ

Психохронография (ПХГ) при помощи эффекта серийной критической частоты мерцания (ЭСКЧМ) предоставляет возможность для изучения посредством перцепции времени общей способности головного мозга человека к перенесению стресса. Ложно-сезональный биоритм 504 мужчин и 219 женщин определялся нисхождением кривых «утомления», «адаптации» или «привыкания». Этот ритм, повидимому, имеет значение при заболеваемости и смертности и заслуживает внимания как в области терапии, так и профилактики.

ДЕЙСТВИЕ КЛОФИБРАТА НА ВОДНЫЙ ОБМЕН БОЛЬНЫХ С ГИПОТАЛАМИЧЕСКИМ И РЕЗИСТЕНТНЫМ К АДГ НЕСАХАРНЫМ ДИАБЕТОМ

Ф. А. ЛАСЛО и Л. ЦАКО

У 9 больных несахарным диабетом, чувствительных к вазопрессину и обладающих запасом АДГ, у одного больного, не обладающего запасом АДГ и у четырех больных, резистентных к АДГ, авторами были изучены изменения водного обмена, возникающие после дачи перорального антилипемического средства: клофибрат (Мисклерон, Химического завода Хиноин). Было установлено, что у 9 больных несахарным диабетом, чувствительных к АДГ, клофибрат понижает выделение мочи, клиренс свободной воды, и повышает осмолярность мочи. У больных несахарным диабетом, чувствительных к вазопрессину, не обладающих запасом АДГ, и у больных, резистентных к АДГ, клофибрат не вызывает изменения диуреза.

По данным исследований авторов клофибрат оказывает АДГ-подобное действие на водный обмен и является средством, хорошо применяемым при лечении больных с гипоталамическим несахарным диабетом, чувствительных к вазопрессину.

О ПАТОЛОГИЧЕСКИХ ИЗМЕНЕНИЯХ ЛЕГКИХ ПРИ ИНТЕРМИТТИРУЮЩЕМ ГИПЕРБАРИЧЕСКОМ ЛЕЧЕНИИ КИСЛОРОДОМ

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LYSOSOMAL ENZYMES IN NEUROLOGICAL AND PSYCHIATRIC CONDITIONS

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Acid phosphatase was estimated in the spinal, cisternal and ventricular CFS of patients suffering from various neurological and psychiatric conditions. Neutral protease activity was also determined in individual cases. The data of more than hundred subjects with well-defined clinical conditions were evaluated. Acid phosphatase was thus ascertained to be present in the CSF, its activity corresponding to approximately one tenth of that in the blood serum. Neutral protease activity was not demonstrable in the CSF of healthy subjects.

A marked increase in acid phosphatase activity was found in benign or malignant tumours of the CNS. Peak values were noted a few hours before death. Normalization of the acid phosphatase level ensued after surgical removal of the tumour. Active protease appeared in the CSF in the case of tumours even if the pH was normal. This raises the possibility of a tumour activity of hitherto unknown type, a long range enzymatic activity in the CNS. Though the cystic fluid of tumours revealed a high phosphatase activity in every case, the cells of the environment, in addition to the tumour tissue itself, are also considered possible sources of the lysosomal enzymes under study.

The lysosomes are assumed to play an important part in the breakdown of own or foreign proteins, as well as of carbohydrates and fats. These subcellular organelles are closely involved in cell division (Allison and Mallucci, 1964), cell differentiation (Farquhar and Palade, 1965) and cell metamorphosis (De Duve and Wattiaux, 1966). On the other hand, under pathologic conditions, liberation of lysosomal enzymes represents the "final common pathway" of cellular autolysis. The enzymes released from the affected cells may cause injury to every tissue (Weissmann, 1967; Lapis, 1968).

Lysosomes are present in the various cell types of the central nervous system (Holtzman, 1969). Certain authors (Gordon et al., 1968) attribute a lysosomal character to the synaptosomes, too, which are assumed to play an essential part in the transmission of stimuli. We have none the less little knowledge of the involvement of the lysosomes and of their enzymes in the various pathological conditions of the central nervous system (Holtzman, 1969). The studies were first focussed on storage diseases including lipidoses and mucopolysaccharidoses (Weissmann, 1964; Koenig, 1969) and were conducted mainly along histological lines, though there have been reports on changes in the lysosomal enzyme levels of the CSF, particularly in inflammatory conditions (Guzowski and Knobloch, 1963; Allen and Reagan,

1964). It has therefore been sought to throw light on the part played by the lysosomes in various neurophsychiatric diseases by studying the CSF for acid phosphatase which has proved a lysosomal indicator enzyme. Estimation of neutral protease was also carried out in individual cases, in view of the possible pathogenetic involvement of this lysosomal enzyme, in addition to its role as an indicator.

Material and methods

CSF obtained by lumbar, cisternal or ventricular puncture was used. In addition to other tests, the total cell count, RBC, and the benzidine reaction were determined in every case. With the exception of acute vascular syndromes and postoperative conditions, cases with a positive benzidine reaction were excluded from evaluation, owing to the acid phosphatase content of the erythrocytes. In this manner we have been able to collect data from more than 100 subjects with well-defined clinical conditions, falling into the following groups: neuroses (serving actually as controls), with no evidence of any organic neurological or of "major" psychiatric disease and with negative CSF-findings; psychoses; epilepsy; cerebral atrophy and chronic vascular lesions; intracranial inflammatory and neuroallergic conditions; acute vascular diseases; intracranial tumours; spinal processes. In view of the small number of cases suitable for evaluation, a further breakdown of the material into individual conditions did not recommend itself.

For the estimation of acid phosphatase, Calbiochem Calsul was used, for the measurement of neutral protease, Azocoll (Calbiochem, Calif.). The original procedures elaborated for serum have been modified to some extent. Use of larger specimens and prolongation of the incubation times allowed to carry out the estimations with the necessary accuracy despite the low lysosomal enzyme activity of the CSF.

For the measurement of acid phosphatase activity the contents of the capsule (p-nitrophenylphosphate and buffer components) were dissolved in 10 ml distilled water, and 1 ml CSF was added to 1 ml of the substrate (pH 5.4). After 60 minutes incubation at 37°C, the reaction was stopped by the addition of 4 ml 0.05 N NaOH. The alkalinity of the medium was indicated by a characteristic change of colour. In the O-minute sample NaOH was measured prior to the addition of CSF. The amount of liberated p-nitrophenol was determined in a Unicam Spectrophotometer in 1 cm-cuvettes on the grounds of the extinction at 405 nm, with the aid of a calibration graph. Enzyme activity was calculated on this basis and expressed, at the first approximation in Bessey—Lowry units, one Bessey—Lowry unit being equal to that amount of acid phosphatase per L serum or CSF which splits from p-nitrophenyl-phosphate 1 mM p-nitrophenol/hr at 37°C (BESSEY et al., 1946). Since the values expressed in this manner were too low in the case of CSF, the tenfold values of the Bessey—Lowry unit were taken arbitrarily as the acid-phosphatase unit for CSF. On these grounds one CSF acid phosphatase unit (CSFAPU) = Bessey—Lowry Unit (BLU)$\times$10.

The serum acid phosphatase level was measured in 0.2 ml samples, by following the instructions valid for Calsul.

For the determinations of neutral protease, 25 mg Azocoll (collagen-bound azo-dye) per test was suspended in 1 ml 0.05 M phosphate buffer, pH 7.5. The mixture of 1 ml substrate and 1 ml CSF was incubated at 37°C for 60 min. The amount of dye liberated by the protease was determined photometrically from the filtrate of the reaction mixture in 1 cm cuvettes at 580 nm. Under the given conditions the enzyme activity is related to the extinction.

Results

The procedure outlined above permits to ascertain the presence of acid phosphatase in the normal CSF, enzyme activity being lower by one order of magnitude than in the serum, that is 0.01 to 0.045 BLU (0.1 to 0.45 CSFAPU) in the CSF as against 0.1 to 0.45 BLU in the serum. In 13 out of 14 cases of the neurosis group, regarded as normal, activity in the spinal CSF was below

0.45 CSFAPU, the findings having been checked by repeated tests in a number of cases. (Fig. 1)

The value for the cisternal and ventricular CSF was generally 0.1 CSFAPU lower than in the spinal CSF.

A substantial rise in the enzyme level of the CSF was found in tumours of the central nervous system, while the serum level usually remained unchanged. Only in one out of 12 cases did the CSF-level fail to attain 0.60

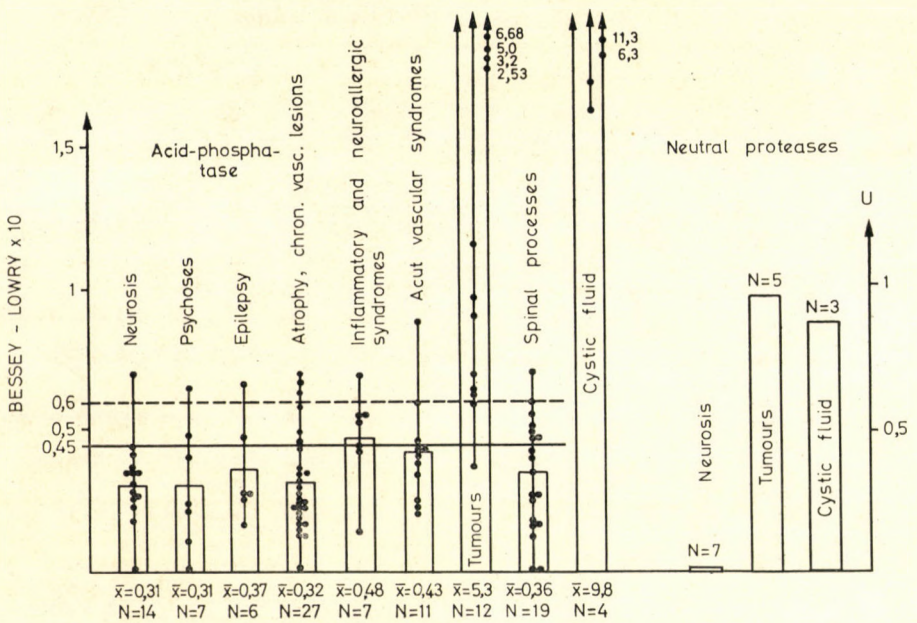

Fig. 1. Acid phosphatase and neutral protease levels in CSF in various neurological and psychiatric conditions. The dots represent values found in the CSF of patients belonging to the individual groups and of the controls (neurosis group). The continuous and the interrupted horizontal lines mark the border range of the normal.

CSFAPU. The rise was demonstrable in benign and malignant tumours alike.

On closer analysis of the cases it was found that, parallel with the growth of the tumour, in other words, with progressive clinical deterioration, the enzyme level in the CSF increases. For instance, activity in the CSF of a 35 year old female patient, T. I., with reticulosarcomatosis, was 0.65 CSFAPU on November 5, 1.1 CSFAPU on November 18, and as high as 6.68 CSFAPU on December 5, prior to death.

In the 37 year old male patient T. L. with a multiform parietal glioblastoma, the enzyme activity was 1.15 CSFAPU on November 21; 25.76 CSFAPU on December 5; and attained the excessive level of 85.1 CSFAPU by the date of his death, December 8. This case has not been included in the

tumour group of Fig. 1 in view of the presence of erythrocytes in the CSF, with similar counts on December 5 and December 8 and a positive benzidine reaction.

After surgical removal of the tumour, a transitory rise of the CSF acid phosphatase level was followed by a decline, as in the case of K. J., a 4 year old boy with an ependymoblastoma. CSF acid phosphatase measured 0.9 CSFAPU preoperatively, 1.72 on the 3rd, 0.8 on the 5th and 0.54 CSFAPU on the 14th postoperative day.

The values found in cystic fluids of tumours were excessively high in every case.

The values obtained in the other groups were inconclusive owing to the small number of cases. It was, however, remarkable that in acute vascular conditions no significant elevation has been found despite the presence of numerous erythrocytes in the CSF and the positivity of the benzidine reaction. In the group of inflammatory and neuroallergic diseases, the values were in excess of 0.45 CSFAPU in 6 out of 7 cases.

In opposition to acid phosphatase, no neutral protease activity was demonstrable in the CSF of healthy subjects. With the exception of the tumour group, it was absent in the other groups, too. However, in the tumour group its activity in the CSF as well as in the cystic fluid of tumours was significantly increased.

Discussion

For the measurement of the lysosomal indicator enzyme, acid phosphatase, Calsul (Calbiochem) has been applied, not only because its use is simple and standardized and the results are therefore reproducible without any difficulty, but also because it contains p-nitrophenylphosphate as substrate which gives a low activity with certain acid phosphatase isoenzymes, in particular with those of erythrocytic or prostatic origin. While the ideal substrate for the prostatic isoenzyme is alpha-naphthylphosphate, the erythrocytic phosphatase gives the highest activities with phenolphthalein phosphate (Babson et al., 1959). This explains why no significant elevation in activity was demonstrable by the use of Calsul even in acute vascular conditions.

On the other hand, as shown in our earlier studies, this substrate lends itself to the measurement of lysosomal phosphatases under normal and abnormal conditions alike (Szilágyi et al., 1973).

Normal blood plasma is known to have a marked protease inhibitory activity. The protease inhibitor complex fails to split proteins but splits synthetic substrates of small molecular weight (Snodgrass, 1970). Since a CSF-protease, to be of any interest, must be measurable even in the presence of large molecular protein substrates, we used Azocoll for these studies.

The procedure applied allowed to determine the presence of acid phosphatase in the CSF of healthy subjects, its enzyme activity being generally below 0.45 CSFAPU. In various pathologic conditions the values ranged between 0.45 and 0.6 CSFAPU without being typical of the individual disease. In contrast, in tumours of the central nervous system, enzyme activity was generally in excess of 0.6 CSFAPU and, as confirmed by repeated measurements, in individual cases it attained 25 to 85 CSFAPU.

The acid phosphatase level was not significantly affected by the presence of erythrocytes. Growth of the tumour and deterioration of the general condition were generally marked by rising activities. On the other hand, surgical removal of the tumour was followed, after a transitory increase, by a tendency of normalization of the originally high values.

Neutral protease was also demonstrable in the CSF of tumour bearing subjects. Neutral proteases originating in the lysosomes seem to be of a pathogenetic significance, at least as far as human leucocytes are concerned (JANOFF and ZELIGS, 1968). This possibility may apply to the present case as well, since the proteases which operate at physiological pH-levels may well affect the protein constituents of the lipoprotein membranes of the various elements of the central nervous system. Moreover, these findings raise the possibility of a hitherto unknown effect of tumours of the central nervous system, namely an enzymatic activity operating at a distance, since the enzymes finding their way into the CSF may well affect nervous structures at a long distance from their sites of release.

Elevation of the lysosomal enzyme levels in the CSF associated with tumours may be connected beyond doubt with the tumour itself or with the injured cells in its neighbourhood. Accordingly, we have to consider various possibilities as regards the source of the increased amount. First of all, they may derive from the very cells of the tumour, a supposition being in agreement with the findings of CLAUSEN (1963) who was able to demonstrate two acid phosphatase isoenzymes (alpha and beta types) in normal cerebral tissue. Meningeomas are marked by a high alpha activity and neurinomas by the presence of three isoenzymes.

Since hypoxia of the central areas of the tumour results in necrobiosis, the necrotizing cells may also represent a potential source of the lysosomal enzymes. The finding of excessive acid phosphatase levels in the cystic fluid of tumours lends support to this possibility.

Finally, the excess of lysosomal enzymes may have its source in the immediate vicinity of the tumour, as suggested by the finding of high acid phosphatase activity demonstrable by histochemical procedures in the reactive glia contiguous to the tumour (SCHIFFER et al., 1967).

The increased enzyme levels noted in inflammatory and neuroallergic conditions may well originate from the lysosomes of the leukocytes.

It is believed that, largely as a result of the high sensitivity of the procedures used for enzyme determination, the present data might provide practical information, adding thus to the laboratory procedures for the diagnostics of neoplastic diseases of the central nervous system.

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STUDY OF THE KINETICS OF IODINE HORMONES BY ABSORPTION SPECTROPHOTOMETRY

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The relationship between the distribution of activity of the individual iodine hormone components and the iodine hormone kinetics of the body was studied. It was also sought to ascertain how many of the two, three or four iodine substituents of the iodine hormone molecule are radioactive. The proportion of active and inactive iodine hormone components of the thyroid was studied by radio-autochromatography and by absorption spectrophotometry. The results may be summed up as follows:

1. The percentage distribution of inactive hormones found during the period of study was constant and in conformity with published evidence.
2. The proportion of active iodine hormone components fluctuated within the range indicative of a dynamic equilibrium. The results may be regarded as informative of the kinetics of production of iodine hormones and of their mobilization.
3. The results support the claim that there is only a single active iodine substituent on the iodine hormone molecule.
4. The individual active iodine hormone components attain the values characteristic of the dynamic equilibrium of inactive iodine hormones by the end of approximately 24 hours.

In the last decades, chromatography combined with radioisotope techniques has become current for the measurement of iodine compounds of thyroid, blood, urine and saliva [2, 5]. Though numerous results have been achieved by this method, still it has to be combined in many cases with non-radioactive physical or chemical procedures of some kind. For instance, distribution of the activity of the individual hormone components does not necessarily reflect their real distribution, particularly during the first hours after administration of the isotope. This makes an additional determination of the distribution of inactive iodine hormones necessary. These measurements are usually carried out by microanalytical procedures requiring special laboratories of meticulous cleanliness.

The application of spectrophotometry [4] in this field not only provides for quantitative determination but also permits to follow the kinetics of hormone-production by the thyroid gland. The joint evidence of spectrophotometry and radiochromatography offers information on the number of radioactive iodine atoms at positions 2, 3 or 4, furthermore on the time elapsing from the administration of ^{131}I until the establishment of a hormonal dynamic equilibrium for the radioactive iodine. (A hormonal dynamic equilibrium

means that the condensation of mono and di-iodotyrosine representing the preliminary phases proceeds at the rate as the release into the circulation of tri-iodothyronine and thyroxine.) This may result in a constant proportion characteristic of the given inactive iodine hormone.

Spectrophotometric measurement of the concentration of iodine hormones and separation of the individual components are based on the fact that on optic induction the four iodine hormones exhibit an ultraviolet light absorption related to the number of benzene rings and of the iodine substituents, and that the maximum of the absorption band shifts to the range of longer waves in accordance with the number of radicals substituted on the benzene rings [1, 6—9].

Methods and Results

In enzymatic homogenisates of rat thyroid glands the released iodine hormones were separated by thin-layer chromatography and the proportion of active and inactive hormones was determined. The activity distribution graphs of the thin-layer chromatograms were submitted to automatic analysis [3]. The percentage distribution of the hormone components was

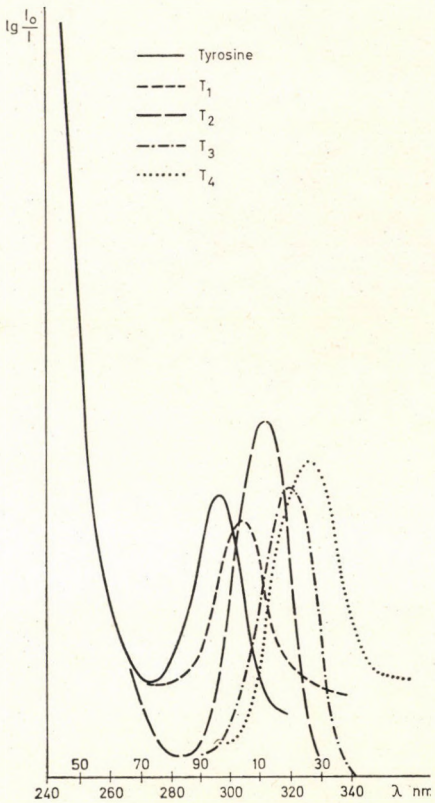

Fig. 1. Absorption of tyrosine and of iodine hormones (Measurements with UNICAM SP-700 apparatus)

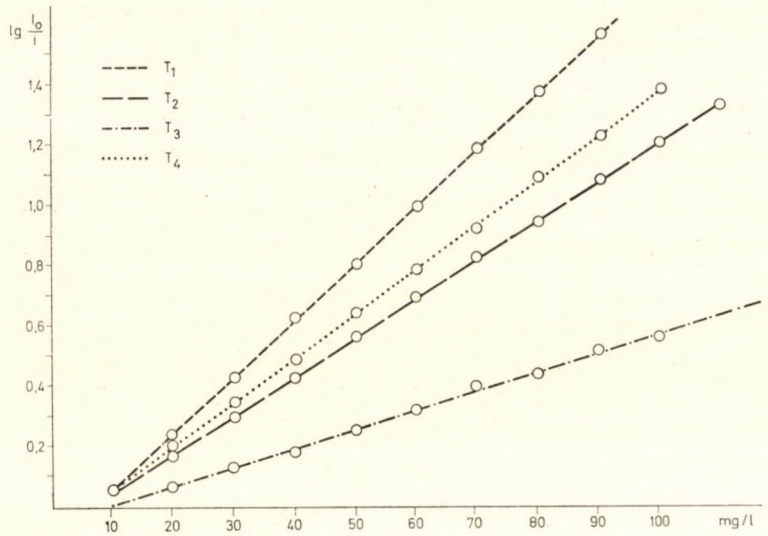

Fig. 2. Correlation between extinction and concentration of iodine hormone solutions, measured at wavelengths of peak absorption

determined by planimetry of the areas of peak activity. The sites of adsorption were identified by radioautography. After delimination of these areas, the components were isolated, dissolved in 2 *n* ammonia and the absorption spectra of the solutions thus obtained were examined with an SF-4 and an UNICAM SP-700 monochromator. The results were similar on both apparatuses.

For the determination of the absorption maxima of the individual components, 10^{-4} mol/L solutions were prepared from each of the four hormones and the absorptions were measured in the range of 250 nm to 360 nm. These results are presented in Fig. 1. As it can be seen, the absorption maximum for the basic compound, p-oxyphenylalanine (tyrosine) is situated at 297 nm, that for mono-iodotyrosine with as single iodine substituent (T_1) at 303 nm, for di-iodotyrosine (T_2, with a single tyrosine ring but with two iodine substituents at positions 3 and 5, at 310 nm. Of the compounds with two tyrosine-rings, tri-iodotyronine (T_3) has its maximum absorption at 320 nm and tetra-iodotyronine (T_4 thyroxine) at 326 nm. The absorption bands are approximately 25 to 30 nm wide. The shift to the right is of the order of 5 to 7 nm if the number of iodine substituents changes by one and of the order of 10 if the rings increase in number.

Since these peaks are close to each other, the absorption ranges overlap, giving a 60 nm wide overall absorption with a maximum at 317 nm for the hormone mixture. From this it follows that the spectrophotometric value of a hormone mixture represents the totality of the extinction values of its constituents, therefore it is not characteristic of any of the components. The increments in absorption at wavelengths shorter than 250 nm must be ascribed to the high absorption of the (2n H_4NOH) solvent.

In the individual fractions of the thyroid homogenisates, the concentrations were always measured at the wavelength corresponding to the absorption maxima in Fig. 1, as follows. The absorptions of iodine hormones of known concentration (10 to 50 mg/L) in ammo nium solution were examined and the results served for the construction of calibration graiphs (Fig. 2). It was to these that the extinction of the hormone solutions of unknown concentraton-was then referred.

Since the procedure covers the totality of thyroid hormones, the distribution of the hormone fractions reflects the state of dynamic equilibrium, if the whole amount of the four hormones in the thyroid is taken as 100%. In order to correlate these values with the radioactive hormone distribution, the percentage of active iodine hormones was also determined. Comparison of these results is seen in Table I where each figure represents the mean of 100 measurements. Fig. 3 gives their graphical representation.

Discussion

From Table I and Fig. 3 it emerges that, as expected, the percentage distribution of the inactive hormones was identical throughout, the values for the individual hormone components being in agreement with those found in the literature [10]. The percentage distribution of the active hormone components fluctuates in the neighbourhood of the values indicative of a dynamic equilibrium, these figures reflecting the kinetics of production of the active components on the one hand and their mobilization from the thyroid, on the other.

In Fig. 3 it can also be seen that at values beyond 24 hours T_2 increases and T_1 decreases. The cause of this might be that di-iodotyrosine, while

Table I

		2 hours	6 hours	24 hours	48 hours	72 hours
T_1	labelled	39.3 ± 1.7	21.3 ± 1.1	24.9 ± 2.2	27.9 ± 0.9	32.1 ± 1.6
	inactive	22.6 ± 1.2	23.5 ± 1.8	23.4 ± 1.3	24.0 ± 1.5	24.9 ± 3.2
T_2	labelled	45.2 ± 1.7	58.5 ± 3.1	52.8 ± 4.5	46.7 ± 2.5	41.4 ± 3.1
	inactive	52.7 ± 1.9	52.8 ± 2.3	51.5 ± 2.8	52.2 ± 2.2	52.7 ± 1.9
T_3	labelled	5.4 ± 0.4	5.4 ± 0.7	5.4 ± 0.3	5.1 ± 0.8	4.3 ± 0.6
	inactive	3.7 ± 0.3	3.9 ± 0.5	3.5 ± 0.2	3.6 ± 0.4	3.6 ± 0.4
T_4	labelled	10.2 ± 1.0	15.5 ± 0.5	17.7 ± 1.1	20.0 ± 0.8	21.9 ± 1.1
	inactive	20.4 ± 0.9	19.8 ± 1.6	20.6 ± 0.8	20.1 ± 0.2	19.2 ± 1.5

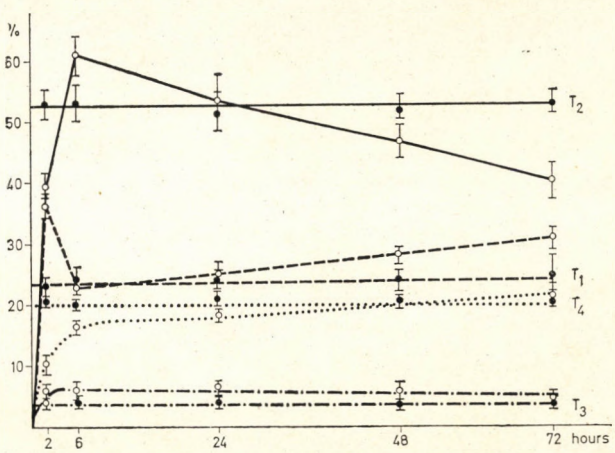

Fig. 3. Time relationships of percentage distribution of inactive and of radio-iodine hormones in the thyroid gland. Full circles refer to inactive, empty circles to active iodine hormones. Each point represents the main of 100 measurements

forming thyroxine in large amounts as a result of condensation, is at the same time involved in the synthesis of T_3. Moreover, it may be converted to mono-iodotyrosine as a result of de-iodination. Closer analysis of the kinetics of this process is in progress.

Likewise with reference to the percentage distribution of active and inactive T_3 and T_4 beyond 24 hours, it must be emphasized that, as shown in Fig. 3, measurements of radio-iodine distribution and those of total hormone distribution gave the same proportion. This confirms the generally accepted but not conclusively proved claim that only one single radioiodine can be incorporated into the same hormone molecule. Were this not so, then, on the grounds of activity distribution, the hormone components with two, three or four iodine substituents would show two-, three- or four-times higher activities.

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CLINICAL AND HISTOPATHOLOGICAL STUDIES OF HUMAN RENAL DISEASE IV. RELATIONSHIPS OF RENAL ARTERIAL FIBROELASTOSIS, GLOMERULONEPHRITIS AND ARTERIAL HYPERTENSION

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It is concluded from histopathological and clinical data of 55 patients with renal disease that fibroelastosis of the renal arteries may develop independently of arterial hypertension, presumably as a result of the primary process. Evolution of the vascular change is closely related to the duration of the process. Though it may occur in any age group, it seems to be prevalent in advanced age. It fails to reveal any relationship with spasms or sclerosis of the retinal vessels. There is no clinical sign which might be regarded as conclusive of the condition of the renal vascular system.

Electron microscopy may reveal changes of the glomerular basement membrane as well as of the epithelial and endothelial cells even in the case of negative light microscopic findings. These changes may be consecutive to earlier glomerulonephritis or to arterial hypertension and account for the presence of proteinuria even if light microscopy fails to reveal any glomerular abnormality.

Intimal fibrosis of the minor renal arteries with lamellar hyperplasia of the elastic membrane is a common finding in arterial hypertension. The relationship between the renovascular abnormalities and arterial hypertension is, however, still controversial. BELL [3] ascribes the intimal thickening of the interlobular arteries to a reduplication of the elastic fibre which, on its part, is closely age-related, but unrelated to arterial hypertension, though enhancing its production. According to BELL [3] the incidence of atherosclerosis increases with advancing age, on the other hand an increased blood pressure is associated with a higher incidence of atherosclerosis. In renal biopsy material of hypertensive subjects obtained in the course of sympathectomy CASTLEMAN and SMITHWICK [5] found the minor renal vessels entirely unaffected in 40% of the cases. This is consistent with the observations of HEPTINSTAL [9]. On the other hand, according to the post-mortem findings reported by ALLEN[1], kidneys of hypertensive subjects hardly ever fail to reveal vascular abnormalities. BALÓ and RÓNA [2] regard lamellar fibroelastosis as a typical vascular pattern in hypertensive disease and chronic glomerulonephritis, and consider the increase in blood pressure as the responsible factor. This interpretation which agrees well with the basic lines of conduct in the therapy of hypertension accompanying chronic nephritis, which seeks to counteract by hypotensive measures the

development of renovascular sclerosis, in other words, of secondary ischaemic damage to the renal parenchyma.

In renal biopsy material marked vascular abnormalities were found in several instances where neither light microscopic, nor immunofluorescent studies had revealed any sign of active glomerulonephritis, biopsy having been indicated on the basis of proteinuria or microscopic haematuria. In order to find an interpretation for these findings, it seemed necessary to review the clinical and histological data of all cases where a biopsy done for the suspicion of glomerulonephritis had confirmed the presence of fibroelastosis or of a lamellar hyperelastosis of the interlobular arteries.

The data of our patient material of 55 cases have been analyzed to answer the following questions.

1. What are the relationships between the renovascular abnormalities, the age of the patients and the duration of their disease?

2. How far are the abnormalities of the renal arteries related to blood pressure?

3. Is there any particular clinical sign which might be regarded as conclusive of vascular nephropathy?

The clinical, histological, immunohistological and electron microscopic methods applied in these studies have been described earlier [4]. In addition to renal biopsy, an immunohistological study was performed in every case and electron microscopy in 25 of the 55 cases.

Results

In 5 of the 55 patients GFR and concentration capacity were reduced but none of them were azotaemic.

The glomerular changes revealed by light microscopy and immunohistologically allowed to classify the patients into the following groups.

a) membraneous glomerulonephritis: 31 cases ;

b) membranoproliferative glomerulonephritis: 4 cases;

c) membraneous glomerulonephritis + pyelonephritis: 2 cases;

d) chronic glomerulonephritis: 3 cases;

e) amyloidosis: 2 cases;

f) focal glomerulosclerosis with immunohistological findings: 7 cases;

g) normal light microscopic glomerular pattern: 6 cases.

The morphology of glomerulonephritis is outside the scope of the present paper which will be confined to the discussion of vascular abnormalities. On the evidence of the light microscopic findings, arteriolar lesions with thickening of the wall due to hyalin deposits and narrowing of the lumen were present in 4 out of 55 cases. In these four cases the major arteries were unaffected. In 8 cases the arteriolar lesions were associated with fibroelastosis of the interlobular

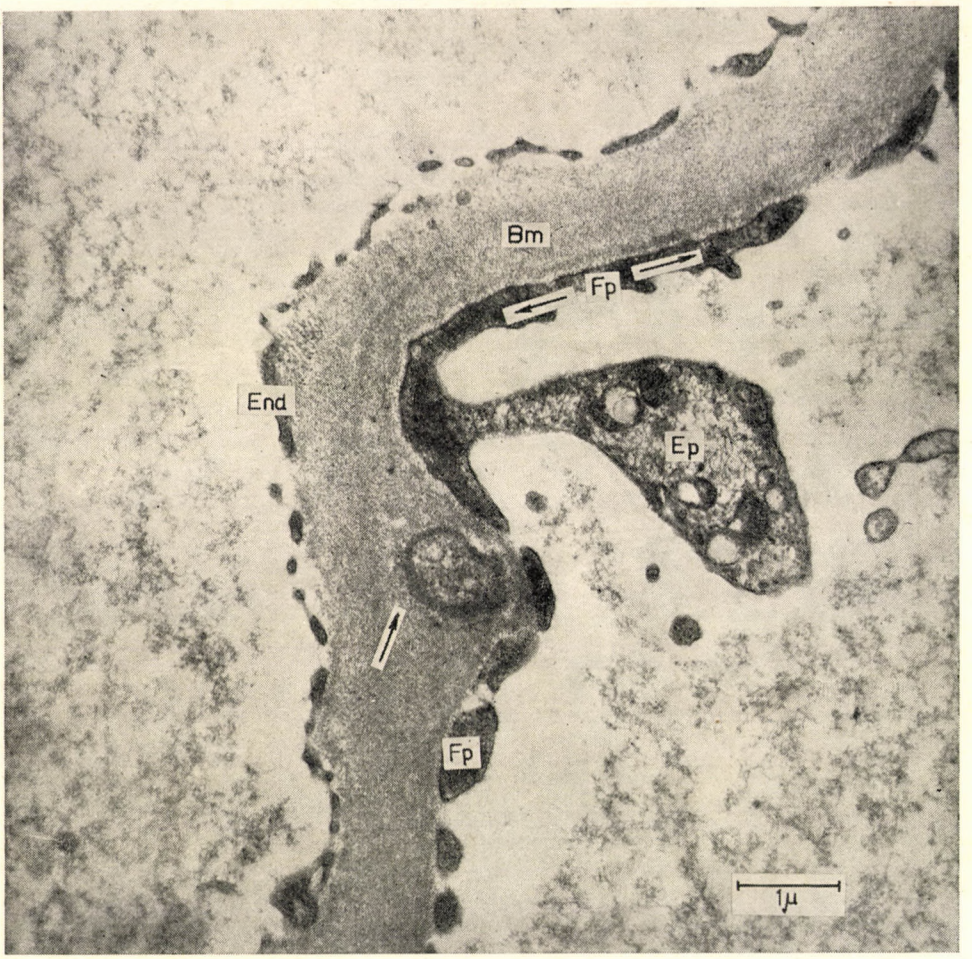

Fig. 1. Electron micrograph. The ground substance of the basement membrane contains a material similar in its structure to epithelial cytoplasm (Ep). Fusion of the foot processes (Fp) of the epithelial cells. (Hitachi HU-10 electron microscope; ×19775)

arteries, while in the rest only fibroelastosis or lamellar hyperelastosis of the interlobular artery was demonstrable. In the cases where glomerulonephritis of the types listed above was accompanied by vascular abnormalities, immunohistology revealed on the basement membrane IgG-, C, and in a number of cases IgM-deposits, while the vessels were free from immune deposits. In the cases where vascular abnormalities were noted without glomerulonephritis, neither the glomeruli nor the vascular walls revealed any immune activity. In 5 of these cases there was light microscopic evidence of a slight glomerular mesangial connective tissue hyperplasia, in the other cases no glomerular change was demonstrable.

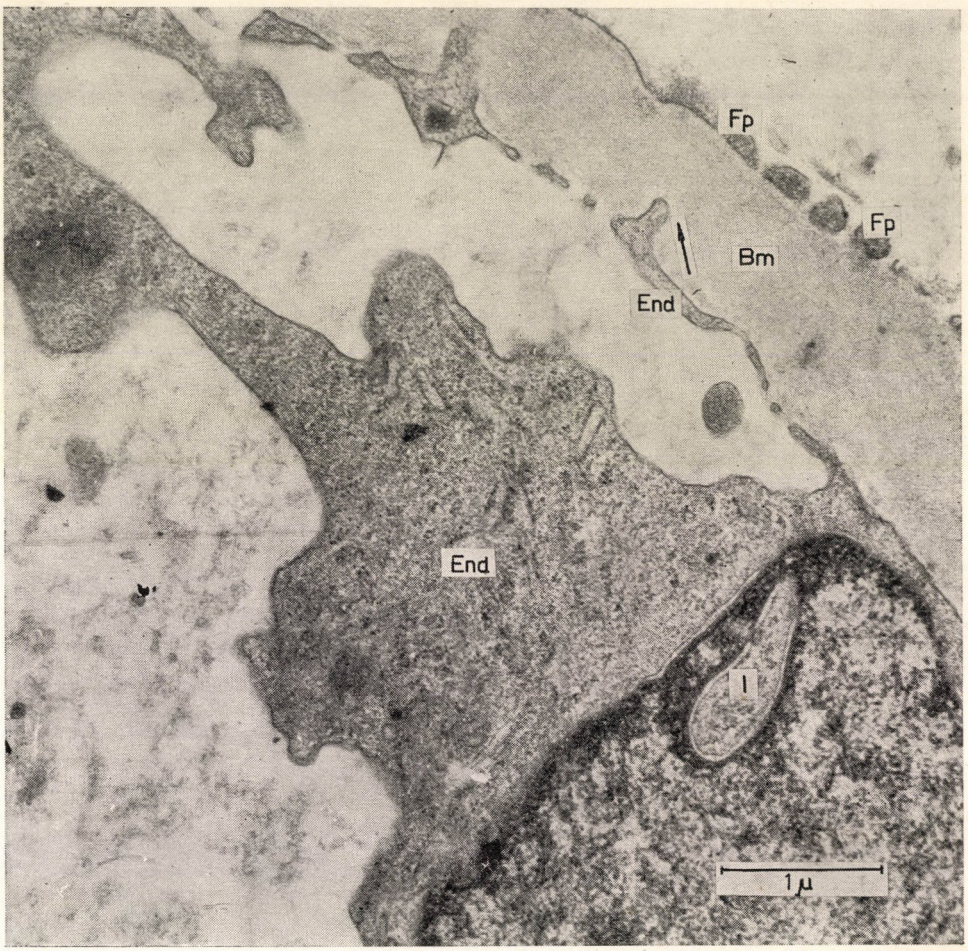

Fig. 2. Electron micrograph. Finger-like projections (→) of an endothelial cell (End) into the basement membrane (Bm). Invagination (I) of the cytoplasm into the nucleus of the endothelial cell. (Hitachi HU-10 electron microscope; ×27720)

The cases in which only vascular changes were present, revealed the following electron microscopic features. The glomerular basement membrane was irregular with uneven thickenings and attenuations. At sites, focal hyperplasia of the mesangial matrix was demonstrable. There were multiple fusions of the foot processes of the epithelial cells, and the basement membrane contained a material reminiscent of epithelial cytoplasm (Fig. 1). At other sites, the endothelial cells were projecting in a finger-like fashion into the basement membrane (Fig. 2) or, occasionally, even penetrating the membrane in its entire thickness (Fig. 3). As a further sign of endothelial activity, intranuclear cytoplasmic invaginations could be noted. The epithelial cells also displayed a strong

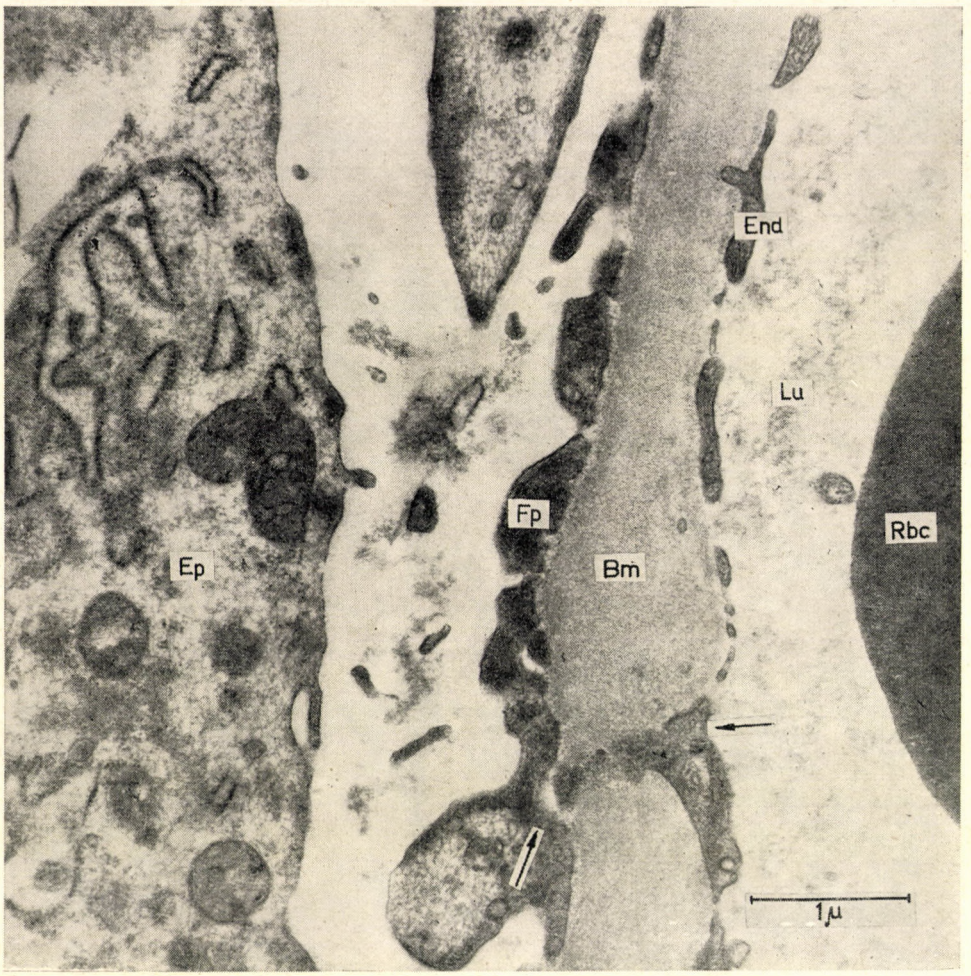

Fig. 3. Electron micrograph. The endothelial cell (End) penetrates deep into the basement membrane (Bm) interrupting its continuity (→). The epithelial cells (Ep) show microvilli and numerous cytoplasmic organelles. (Hitachi HU-10 electron microscope; ×27720)

activity as reflected by the formation of microvilli and the presence of numerous intracytoplasmic organelles. In the interlobular artery the cytoplasm of the smooth muscle cells contained sporadic lipid droplets, there was accumulation of a substance similar in its electron microscopic structure to the basement membrane (Fig. 4). The arteriolar walls revealed an electron-dense granular material in addition to lipid droplets (Fig. 5).

One of the interesting ultramicroscopic findings was the penetration of epithelial and endothelial cells into the basement membrane. Changes of this kind have been first described by SPIRO [12] who pointed to a causal relationship between proteinuria and the notches on the basement membrane pro-

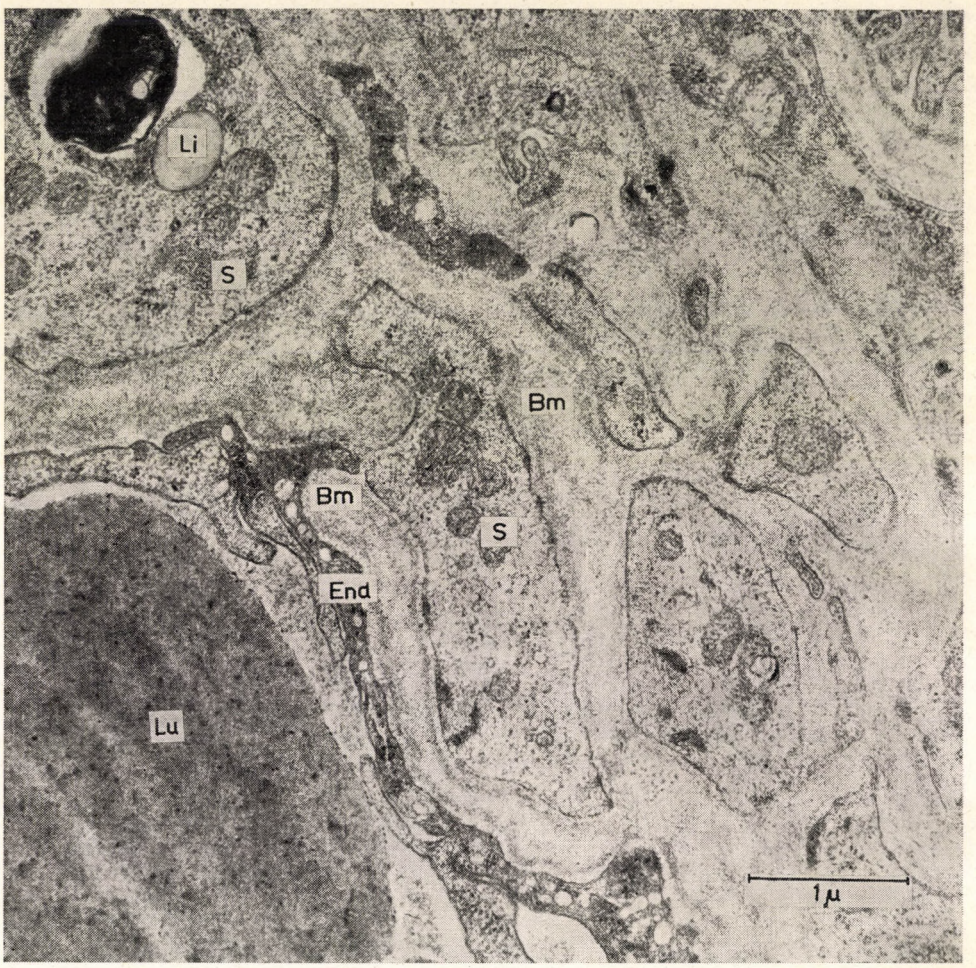

Fig. 4. Electron micrograph. The interlobular artery shows accumulation of a substance similar in its structure to the basement membrane (Bm). In the cytoplasm of smooth muscle cells (S) there are sporadic lipid droplets (Li). (Hitachi HU-10 electron microscope; ×27720)

duced by the foot processes of the epithelial cells. CHURG et al. [6] described similar changes in unclassified renal diseases. LYNN [10] induced arterial hypertension after ligation of one renal artery with contralateral nephrectomy or by bilateral arterial constriction, and found that epithelial and endothelial cells were projecting their pseudopodia into the basement membrane as early as 30 min after starting the experiment. There is strong evidence of causal relationships between proteinuria and these electron microscopic changes. Since, in addition to these findings, hyperplasia of the mesangial matrix and fusion of the foot processes are also present in a number of cases, these abnormalities

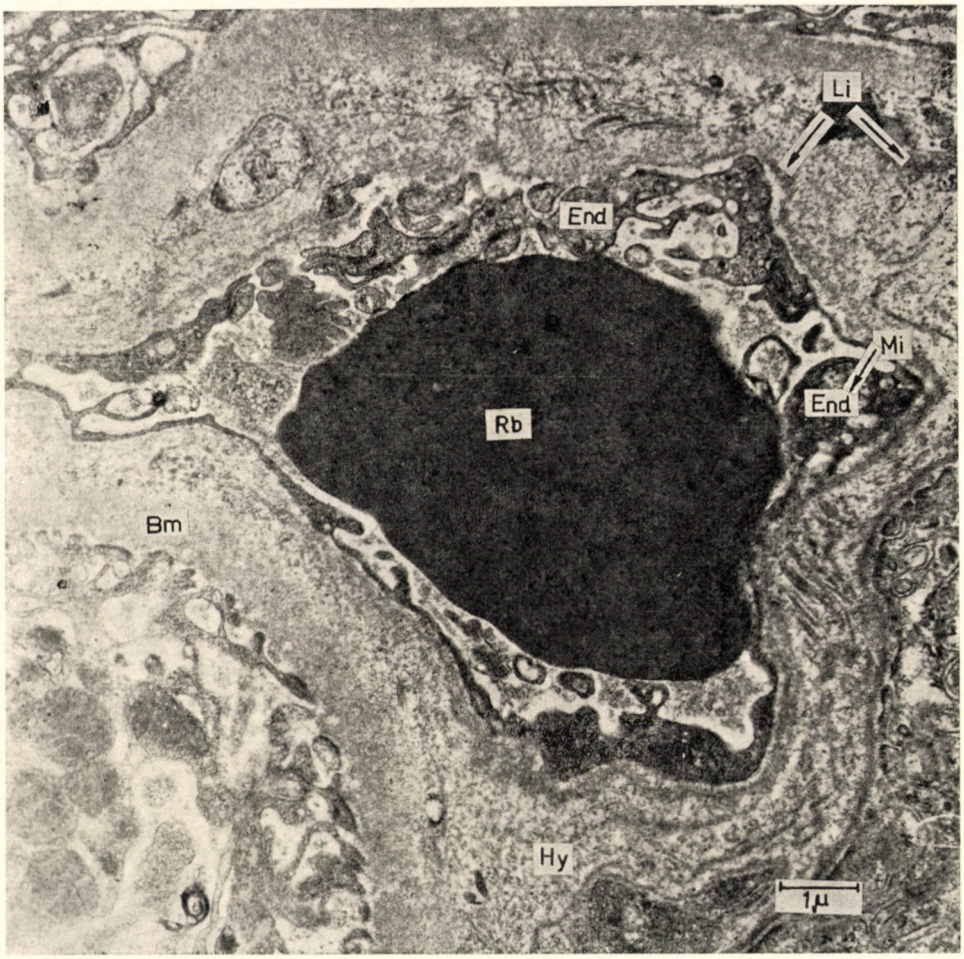

Fig. 5. Electron microscopic finding of cross-section of a renal arteriole. The lumen is packed with erythrocytes (Rb). The endothel cells (End) have preserved their structure. There are subendothelial electron-dense granules (Hy) and lipid-droplets (Li). (Hitachi HU-10 electron microscope; ×13230)

may well represent residual changes after earlier glomerulonephritis, a possibility suggested by two of our observations where glomerulonephritis which had been confirmed by the first biopsy was no longer demonstrable by the second biopsy, performed after the completion of immunosuppressive treatment, though residual vascular abnormalities were still present. Moreover, 4 out of 6 patients where light microscopic evidence was confined to vascular lesions, in the history there were indications of acute glomerulonephritis and of hypertensive episodes. However, for these changes the hypertension may have been responsible, similarly as in LYNN's experiments [10].

It has been expected to clarify the issues raised in the introduction by confronting clinical observations with pathological evidence. The data thus obtained (see Tables) pointed to the following relationships.

1. Fibroelastosis of the renal arteries may occur at any age (Table I). It was, however, remarkable that when the process is traceable to its beginning,

Table I

Indications of renal disease and retinal changes in the history, in the various age-groups

Age group years	Number of cases		Time elapsed since the earliest sign of renal disease years	Occurrence of hypertension			Retinal changes
	males + females	total		limited to antecedents earlier than 1 year back	limited to time of biopsy	persistent	
10—20	3 + 3	6	3.2	—	1	1	2
21—30	1 + 13	14	4.1	3	3	5	5
31—40	4 + 10	14	5.0	2	3	2	7
41—50	7 + 7	14	4.7	2	2	5	11
51—60	4 + 3	7	1.3	—	1	2	6
Total	19 + 36	55		7	10	15	31

Note: In the history, the earliest event indicative of renal disease was acute nephritis in 7, toxaemia of pregnancy in 7 cases. In all other cases, detection of renal disease was incidental, in connection with screening or other investigations.

it is unusually of long duration, except in the advanced age groups where it is of fairly recent origin. In reality, the process must have dated back earlier than indicated by the figures in the Tables. It was actually in 14 cases that we were able to trace its origin with a reasonable degree of probability: in 7 cases it was acute nephritis, in 7 toxaemia of pregnancy which had led to the diagnosis of renal disease. In all the other cases its discovery was incidental, as a result of screening or of routine investigation for some other reason. Therefore the onset must have been latent.

2. Renal arterial changes were found even in the presence of a normal blood pressure at the time of biopsy (Table II). It is true that in a large number of patients who were normotensive at the time of study there had been either hypertensive episodes in the past or other events (acute nephritis, toxaemia of pregnancy) which may have been associated with hypertension.

As it can be seen from Tables I and II, changes of the renal arteries need not be associated with spasm or sclerosis of the retinal vessels. On the other hand, as clearly indicated by the figures, the prevalence of these lesions is closely related to age and to the severity of hypertension.

3. In the present series, renovascular lesions associated with the clinical syndrome of chronic glomerulonephritis were of a comparatively low incidence

Table II

Relationships between indications of renal disease, retinal changes and blood pressure in the history

Blood pressure (mmHg) at time of biopsy	Number of cases	Indication of renal disease in the case history			Retinopathy at time of biopsy
		hypertension	acute nephritis	toxaemia of pregnancy	
Under 140/95	30	7	2	3	11
140/95—180/110	18	8	5	1	14
Over 180/110	7	7	—	3	6
Total	55	22	7	7	31

Table III

Clinical features and age

Clinical features		Number of cases		Mean age
proteinuria	erythro-cyturia	females	males	years
oedema	hypertension			
—	—	1	0	32.0
—	+			
+	—	2	5	30.4
—	—			
—	+	0	1	41.0
—	—			
+	+	3	7	38.5
—	—			
+	—	2	2	49.0
+	—			
+	—	4	7	35.4
—	+			
+	+	1	5	34.0
+	—			
+	+	6	9	38.7
+	+			

(Table III). Most cases were clinically heterogeneous and oligosymptomatic; isolated proteinuria or haematuria, eventually arterial hypertension being the commonest features, but the nephrotic syndrome was also represented by some cases.

Discussion

On the evidence of corrosion studies of kidneys from subjects dead with renal failure, GÖMÖRI et al. [7] claimed arterial hypertension to be the factor responsible for the narrowing of major (arcuate and interlobular) renal arteries and advocated hypotensive treatment in renal hypertension. On the other hand, they found no correlation between the duration of hypertension and the severity of lesions of the renal arteries. In earlier studies, MORITZ and OLDT [11] noted renovascular sclerosis in approximately 12% of normotensive cases. In pyelonephritic kidneys, SZINAY et al. [13] found lamellar hyperelastosis regardless of the blood pressure almost exclusively in cases where the renal parenchyma was gravely affected, the lesions being of focal pattern. These findings were regarded by the authors as compatible with the possibility that the vascular changes were due to the parenchymal injury. In their view, in the areas affected by inflammatory or toxic processes, even normal blood pressure may give rise to lamellar hyperelastosis and high blood pressure may precipitate its production, it being assumed that the tone of the "weakened" arterial wall becomes inadequate to resist the pressure in its lumen, a state enhancing the development of initial fibrosis. As emphasized by HEPTINSTAL [9], glomerular changes are of two types, the first resulting from nephritis and the second from the vascular lesions associated with arterial hypertension, but differentiation between the two types is not always possible.

In the light of these considerations we feel justified in interpreting our findings as follows. The arterial changes in glomerulonephritis are unrelated to hypertension. The responsible factor is the glomerulonephritis itself which affects the arterial wall to a degree that a normal or a transiently elevated blood pressure may be sufficient for injuring the internal elastic membrane. In old age, atherosclerosis may represent a predisposing factor, a possibility supported by the observation that in the present cases of renovascular lesion in the age group between 50 and 60 years, the renal disease was of recent origin. The lesions may arise even after the healing of glomerulonephritis and follow a progressive course in the presence of normal, immunohistologically inactive glomeruli. This would actually mean that in healed cases the glomerulonephritis may well change its pattern and assume vascular features. This is what seems to have happened in two of our cases where the vascular abnormality ensued after a successful immunosuppressive treatment of glomerulonephritis, and in two further cases of immunohistologically inactive glomerulopathy associated with proteinuria, where during an observation period of two years we witnessed a progressive rise in blood pressure parallel with the development of angiopathy. It is common knowledge that interstitial round cell infiltration, fibrosis and impairment of blood supply associated with persistent glomerulonephritis are bound to result sooner or later in tubular

atrophy. The vascular lesions are secondary to the glomerular process and are present even in the absence of hypertension. After the healing of glomerulonephritis they may represent the actual renal disease. It should be borne in mind that glomerulonephritis affects the nephron, the interstitial tissue and the vascular bed, in brief the kidney as a whole.

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RENAL HYPERTENSION INDUCED BY A NEW PROCEDURE IN THE RAT

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A new procedure has been developed for the induction of renal hypertension in the rat: the renal artery of one side was ligated without interference with the opposite kidney. During an observation period extending to 30 weeks permanent elevation of blood pressure by approximately 55 mmHg, together with cardiac and renal hypertropy has been found in rats under one year of age.

Studies of the renal implication of systemic hypertension started with the fundamental experiments of GOLDBLATT et al. (1934). They restricted renal flow by applying an adjustable clamp to one renal artery in the dog and found this to cause a slight transitory rise of systemic blood pressure. No permanent elevation of blood pressure was obtained unless both renal arteries were constricted or, in the case of unilateral clamping, the opposite kidney was removed. Elevation of blood pressure was related to the degree of constriction. Though it was clearly demonstrated by these experiments that arterial hypertension may be of renal origin, they provided no analogy whatsoever to human clinical syndromes, since in the animals no permanent hypertension ensued in the presence of preserved renal function (GOLDBLATT, 1937; BERMAN, 1971). However, in the last decade several workers have succeeded in producing permanent hypertension by unilateral intervention, in particular FRANK (1963) in monkeys, BOUNOUS and SCHUMACHER (1962), BRATTSTRÖM and KALKOFF (1969), HAYDUK et al. (1970) in dogs, BLAIR-WEST et al. (1968) in sheep. The present authors (FEKETE, 1970) have noted hypertension over several years in dogs after complete ligation of one renal artery without interference with the opposite kidney.

Rats are more liable to develop chronic experimental hypertension after intervention on one kidney than are other species. WILSON and BYROM (1939) induced chronic hypertension in rats by means of an adjustable clamp applied to one renal artery, leaving the other kidney unaffected. GOLDBLATT, KAHN and HANZAL (1939) applied the arterial clamp to the aorta between the origin of the two renal arteries in rats, thus leaving the kidney of one side unaffected.

We have been able to induce chronic hypertension in rats by ligation of one renal artery without affecting the opposite kidney. While the adjustable clamp permits no full control of renal flow, the present procedure has the

advantage of restricting renal blood supply to the utmost, if not blocking it altogether, moreover, the opposite, unmanipulated kidney can be studied as long as necessary.

In order to ascertain whether our procedure is suitable for the investigation of renovascular hypertension we undertook serial studies of the features of the condition thus produced which also allowed to examine the implications of species specificity by comparing the results with those obtained in dogs. The data collected during an observation period of 30 weeks in respect of blood pressure and of cardiac and renal weight in rats after complete ligation of one renal artery have been summed up in the present study, the results being referred to the corresponding data of unoperated rats and of animals submitted to unilateral nephrectomy.

Material and method

Home-bred female rats weighing between 160 and 230 g were studied. Prior to the study and throughout its entire course they were kept on synthetic rat food containing approximately 150 mEq/kg sodium, water being allowed ad libitum.

Group I. *Controls:* 16 normal rats with 229 g mean body weight were anaesthetised with ether. Arterial blood pressure was measured by a mercury manometer inserted into a carotid artery. Then anaesthesia was deepened until death had ensued, body, heart and kidney weight was recorded, the heart being excised at the proximal origin of the large vessels and the kidneys being decapsulated and isolated at the hilus.

Group II. *Unilateral renal artery ligation.* In 47 rats of 175 g mean body weight under ether anaesthesia the left renal artery was ligated from midline approach and the wound was closed. At 2, 6, 13, 16 and 30 weeks, blood pressure was measured under intraperitoneal pentobarbital (37.5 mg/kg) anaesthesia, then the organs were removed and weighed.

Group III. *Unilateral nephrectomy.* In 24 rats of 177 g mean body weight, under ether anaesthesia left nephrectomy was performed from the midline approach and the wound was closed. At 6, 13 and 30 weeks after the intervention, under pentobarbital anaesthesia blood pressure and organ weights were determined.

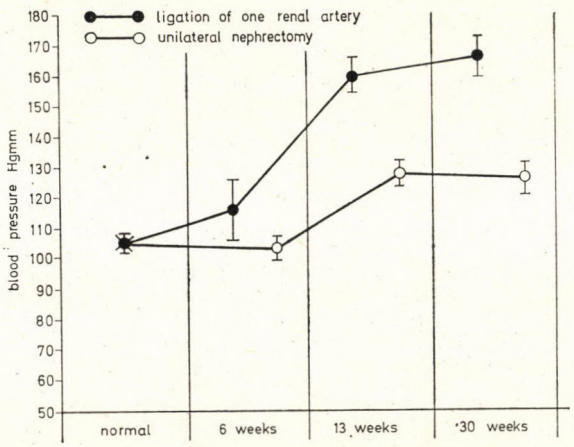

Fig. 1. Blood pressure in rats during an observation period of 30 weeks after ligation of one renal artery (●——●) and after unilateral nephrectomy (o—o) compared with values for the controls

Results

Results have been summarized in Table I and Figs 1, 2 and 3. Statistical evaluation was done with the two-sample *t* test and regression analysis.

1a) Unilateral ligation of one renal artery without affecting the opposite kidney was followed by persisting hypertension. A slight rise in blood pressure

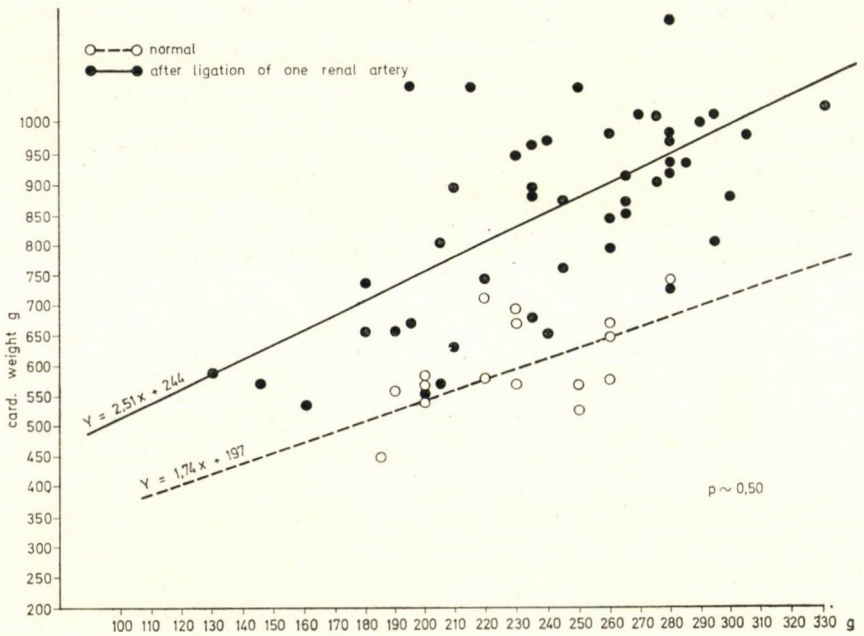

Fig. 2. Cardiac weight referred to body weight in normal (o- - -o) and hypertensive (●——●) rats

was demonstrable as early as 2 to 6 weeks after the intervention. By the end of the 13th postoperative week, the mean increase attained +55 mmHg and blood pressure remained high throughout ($p < 0.001$) (Fig. 1).

1b) Increased cardiac weight was demonstrable over the entire 30 weeks of observation. The ratio heart weight to body weight was 0.26% in the controls, while it rose to 0.35% in the operated rats by the end of the study ($p < 0.001$) (Table I).

1c) Weight of the unoperated kidney increased from 0.39% of body weight in the controls to 0.57% of body weight in the test rats ($p < 0.001$).

1d) For closer evaluation of the changes in heart, and unoperated kidney weight, the corresponding values for control animals belonging to different body weight levels have been compared with those of hypertensive rats of the same body weight and the correlations between the regression lines derived from these data have been analyzed (Figs 2, 3). On these grounds, in hypertension consequent upon ligation of one renal artery, heart and unoperated

Table I

Blood pressure, total body, heart and kidney weight in normal rats, in rats after ligation of one renal artery and after unilateral nephrectomy

Animal group		Number	Blood pressure mm Hg	Body weight g	Cardiac weight mm Hg	Cardiac weight (per cent of body weight)	Weight of one kidney mg	Renal weight (per cent of body weight)	Weight of ligated kidney mg	Renal weight (per cent of body weight)
Control		16	105±2 n = 13	229±7	595±21	0.26 ± 0.08	910±39 n = 32	0.39 ± 0.01 n = 32	—	—
2 weeks	after liga-	6	114±6	168±11 (181±7)*	619±29	0.38 ± 0.01 d	951±44	0.58 ± 0.03 d	401±79	0.24 ± 0.04
6 weeks	tion of	7	116±10	208±6 (173±4)*	664±33	0.32 ± 0.02 d	1121±44	0.54 ± 0.02 d	128±17	0.06 ± 0.01
13 weeks	left renal	7	160±6 n = 13 d	247±6 (167±5)*	851±37	0.34 ± 0.02 d	1154±53	0.51 ± 0.01 d	123±9	0.05 ± 0.002
16 weeks	ar- tery	9	159±7 n = 8 d	264±10 (165±5)*	951±24	0.36 ± 0.02 d	1570±157	0.60 ± 0.01 d	111±6	0.04±0.0001
30 weeks		18	167±8 n = 11 d	269±8 (179±5)*	964±34 d	0.37 ± 0.03 d	1644±67 d	0.60 ± 0.03 d	93±9	0.03 ± 0.003
6 weeks	after left	6	103±5	197±7 (176±5)*	586±13	0.30 ± 0.02 b	1046±62	0.53 ± 0.03 d	—	—
13 weeks	ne- phrec-	6	128±5 d	257±8 (172±6)*	821±35	0.32 ± 0.02 d	1413±60	0.55 ± 0.02 d	—	—
30 weeks	tomy	9	126±6 d	257±10 (183±6)*	923±56 d	0.34 ± 0.02 d	1434±139 d	0.52 ± 0.05 d	—	—

a = $p < 0.05$; b = $p < 0.02$; c = $p < 0.01$; d = $p < 0.001$
* = body weight at 0 day

kidney weight proved to be in excess of the values predicted on the basis of the increase in body weight, as indicated by an increase in the regression coefficient. In kidney weight, there was a significant deviation ($p < 0.05$) between the coefficients of the two regression lines, whereas for heart weight this deviation was not significant statistically.

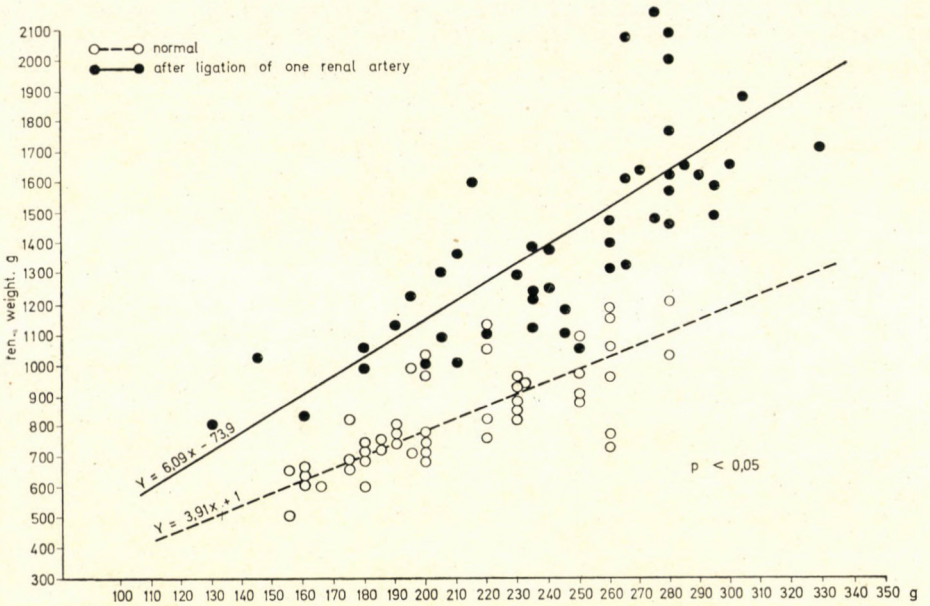

Fig. 3. Renal weight referred to body weight in normal (o- - -o) and hypertensive (● ——●) rats

2a) A slight (mean + 25 mmHg) but statistically significant increase in blood pressure ensued in the animals with unilateral nephrectomy, from the 13th postoperative week onward ($p < 0.001$) (Fig. 1). On the other hand, these values were significantly lower than those found after ligation of the renal artery ($p < 0.001$).

2b) At all times of measurement over the entire period of 30 weeks, after unilateral nephrectomy increased cardiac weights attaining 0.32% of body weight, as against 0.26% in the controls ($p < 0.001$), were demonstrable (Table I).

2c) An increase in unoperated kidney weight attaining 0.53% of body weight, as against 0.39% in the controls, was also demonstrable ($p < 0.001$).

Discussion

Production and stabilization of arterial hypertension ensued after ligation of one renal artery in rats. In addition to its simplicity, this unilateral

intervention has the advantage of leaving the opposite kidney unmanipulated and allowing its continuous study in the state of hypertension.

One of the mean features of the hypertensive condition thus produced is cardiac hypertrophy. As pointed out by DONALDSON (1924), in the Wistar rat growth of the heart and the kidney is rapid in early life, while after sexual maturation (beyond the second month of life) the rate of growth of these organs keeps pace with that of the entire body, though absolute weights may vary according to species. Left ventricular hypertrophy has been noted in association with hypertension in rats by WILSON and BYROM (1939). GROLLMAN and HALPERT (1949) found the increase in cardiac weight to be related to the blood pressure level.

In the present study, in rats of 229 g mean body weight mean heart weight was 595 $\pm$ 21 mg (252 mg/100 g body weight). In the animals with one renal artery ligated similar body weights were registered 13 weeks after the production of hypertension (Table I) and the heart weighed 851 $\pm$ 37 mg (345 mg/100 g body weight) corresponding to an absolute increase in weight of the order of 40%. Cardiac hypertrophy of a similar degree was found in renal hypertension by MASSON, KASHII and PANISSET (1964).

The findings in respect of unoperated kidney weight in chronic hypertension are inconsistent. ARONSON and SAMPSON (1951), MASSON, KASHII and PANISSET (1964), SCHLEGEL and OKAMOTO (1961), HAYSLETT, KASHGARIAN and EPSTEIN (1967), MERTZ and WEISS (1971) observed a compensatory hypertrophy, while other workers, in particular OMAE and MASSON (1960), BRAUN-MENENDEZ (1958), GLAZIER and LOMBARDO (1959), GUYTON and COLEMAN (1969) not only failed to note any increase in unmanipulated kidney weight but even demonstrated grave vascular lesions characteristic of nephrosclerosis. In the view of MERTZ and WEISS (1971), the disposition of the laboratory animal to develop renal hypertrophy depends on its age. In 2-or 3-year-old rats, hyperplasia is negligible while in young animals it is a common occurrence.

The inconsistency of data may have two possible sources. First, the various authors studied laboratory animals of various ages, and, second, the duration of hypertension was different when the results were registered. Therefore, the results may have been influenced by the regenerative capacity of young tissues and by the duration of the hypertensive state.

In our rats of 229 g mean body weight being in possession of both kidneys, 800 mg renal tissue may be calculated for 100 g body weight, each kidney weighing approximately 910 $\pm$ 39 mg. Ligation of one renal artery resulted in an atrophy of the ischaemic and a hypertrophy of the unmanipulated, kidney. In hypertensive rats matched for body weight (e.g. 13 weeks after the intervention) the weight of the unmanipulated kidney was 1253 $\pm$ 53 mg, showing an increase of approximately 40%. These data are in agreement, even in respect of the numerical data, with those of MASON, KASHII and PANISSET

(1964) according to which the atrophic kidney weighed 330 mg and the hypertrophic kidney 938 mg two weeks after production of hypertension. On these grounds, 500 to 600 mg renal tissue may be thus predicted for each 100 g body weight as a result of hypertrophy of the unoperated kidney in this type of hypertension. According to a rough estimate solely on the ground of hypertrophy, this renal substrate provides for 60 to 70% of the function of both kidneys.

After unilateral nephrectomy a mild but statistically significant rise in blood pressure, slight cardiac and significant renal hypertrophy were demonstrable. In earlier studies we found similar changes in the same parameters of dogs subjected to the same intervention. Permanent systemic hypertension consequent upon unilateral nephrectomy was found by GROLLMAN, MUIRHEAD and VANATTA (1949) in dogs, by GROLLMAN (1948) in rats, the condition being associated with hypertrophy of heart and kidney. Nephrectomy in 1 to 2-year-old rats was followed by a rise in blood pressure from 100 to 150 mmHg in 57% and above this level in 20% of the animals, blood pressure remaining unaffected in 23% (GROLLMAN and HALPERT, 1949). As pointed out by MERTZ and WEISS (1971), hypertrophy of approximately 70% will invariably develop in a few weeks after nephrectomy, provided the remaining kidney is normal morphologically. SABIN (1948), HAYSLETT, KASHGARIAN and EPSTEIN (1967) regard the compensatory hypertrophy of the remaining kidney as a crucial factor in preventing the persistence of hypertension. The fact that in the present study elevation of blood pressure consequent upon unilateral nephrectomy was of no significant degree is interpreted as being due to this compensatory mechanism.

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PLASMA, BLOOD AND TOTAL WATER VOLUME IN EXPERIMENTAL RENAL HYPERTENSION IN THE RAT

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Permanent systemic hypertension was induced in rats by complete ligation of one renal artery without interference with the other kidney. A reduction in plasma and blood volume without alteration of the total water volume was demonstrable 13 weeks as well as 30 to 37 weeks after the production of hypertension.

It has been reported earlier that a state of permanent hypertension was produced in rats by ligation of one renal artery, the opposite kidney having been kept free from any manipulation (FEKETE and TARJÁN, 1972). In opposition to the conventional methods making use of adjustable clamps applied to the renal artery where the renal blood supply remains incalculable, our procedure restricts arterial blood supply to the kidney to a minimum, if not blocking it altogether. It has moreover the advantage that the opposite, unmanipulated kidney can be studied as long as necessary. In order to ascertain whether this model was suitable for the study of human renovascular hypertension we had undertaken serial studies of the features of the hypertensive state thus produced. It could be observed that ligation of one renal artery resulted in (1) a substantial rise in blood pressure and in its stabilization at high levels, (2) cardiac hypertrophy, (3) a marked hypertrophy of the unmanipulated kidney.

It is well known that hypertension affects various mechanisms, in the first place those of renal origin which may lead to a shift in the electrolyte and fluid balance. This gives obvious interest to studies of the parameters involved in these mechanisms.

The present paper is summarizing the results of our studies in rats relating to plasma, blood and total blody water volume in hypertension induced by unilateral ligation of the renal artery, the observations extending to a period of 37 weeks. The results have been compared to corresponding data derived from normal rats.

Material and methods

Home-bred female rats weighing between 160 and 230 g were studied. Before and during the period of study they were kept on synthetic rat chow containing 150 mEq/kg sodium, water being allowed ad libidum.

Intervention. In 47 normal rats (mean body weight 196 g) under ether anaesthesia and from midline approach the left renal artery was ligated and the wound was closed. 6 to 8, 13, then 30 to 37 weeks after the intervention, carotid blood pressure was measured with a mercury manometer under pentobarbital (37.5 mg/kg) anaesthesia in successive animal groups and at the same time the fluid compartments were studied. The intravascular and total body fluid compartments as also the dry substance were measured in separate animal groups.

Estimation of plasma and blood volume. After measurement of blood pressure a plastic cannula attached to the tip of an injection needle which was connected with a tuberculin syringe of 1 ml was inserted into the jugular vein on both sides, one serving for the injection of 1 ml of 0.2% Evans blue (T-1824), the other for the withdrawal of 0.6 ml blood samples at intervals of 5, 10 and 15 min after the injection. In the samples, the haematocrit value was determined by a Hawskley microhaematocrit centrifuge. For the replacement of fluid volume lost at sampling, physiological saline was administered. After centrifugation of the samples and dilution of plasma, the plasma extinction values read on an ELKO III photoelectric colorimeter were extrapolated for 0 minute. From the plasma volume thus calculated, total blood volume was computed with reference to the haematocrit value (GREGERSEN 1944; BÁLINT, 1958, 1962). The values were expressed in terms of per cent of body weight too.

Estimation of total body water volume. After measurement of blood pressure and sacrifice of the animals the weighed carcasses were dried to constant weight at 105°C (MITCHELL et al., 1945), the animals having been previously stripped of their skin and spread out on aluminium foil. After dehydration (14 to 16 hours) the carcasses were weighed again. The values for dry substance and fluid volume were expressed in per cents of body weight.

Results

The fluid compartments of hypertensive rats have been compared to those of normotensive controls. The hypertensive animals have been studied at fixed intervals which had been determined by the results of our previous experiments according to which before the 6th week after the intervention, hypertension was not yet demonstrable but could be confirmed beyond doubt from the 13th week onward.

Results have been summed up in Table I and Fig. 1. For statistical evaluation, the two-sample t test was used.

1. In the first group of 20 animals (mean body weight, 208 g) and in the second group of 17 animals (mean body weight, 219 g), thus in a total of 37 animals, mean blood pressure was 105 ± 2 mmHg. In the first group, plasma volume was $5.07 \pm 0.08\%$; mean blood volume, with reference to the 42% haematocrit value, was $8.73 \pm 0.17\%$ of body weight. In the other normotensive group of 17 animals, the fluid content was $63.9 \pm 0.8\%$ and the dry residue, $36.1 \pm 0.9\%$.

2. Between the 6th and 8th week after the intervention a slight rise in blood pressure to 116 ± 10 mmHg was demonstrable. Plasma and blood volumes were similar to those found in the control groups. At this phase of the experiment the total body water volume was not measured (Table I).

3. At 13 weeks after the intervention, there occurred a statistically significant rise in blood pressure. In the group of 15 rats (blood pressure, 160 ± 6 mmHg) plasma volume was $4.52 \pm 0.10\%$ and blood volume, computed with reference to the haematocrit value of 41%, was $7.72 \pm 0.15\%$ of

body weight, both parameters being significantly reduced as compared with the controls ($p < 0.001$). In the other hypertensive group of 12 rats (blood pressure 176 ± 2 mmHg) body water volume was 63.3 ± 0.8% and the dry substance, 36.7 ± 0.6% of body weight, similarly as in the controls.

Table I

Plasma and blood volume, fluid content and dry substance in rats with hypertension consequent upon ligation of one renal artery, and in normal controls

	Controls	6 to 8 weeks after ligation	13 weeks of one renal artery	30 to 37 weeks of one renal artery
Body weigh(g)	208 ± 10 219 ± 4	240 ± 9*	244 ± 6 256 ± 6	263 ± 14 286 ± 8
Number of animals (n)	20 17	9	15 12	5 6
Blood pressure mmHg	105 ± 2	116 ± 10	160 ± 6[d] 176 ± 2[d]	167 ± 8[d]
Plasma volume ml	10.89 ± 0.56	11.95 ± 0.35	11.05 ± 0.37	11.93 ± 0.75
Plasma volume (per cent of body weight)	5.07 ± 0.08	5.04 ± 0.23	4.52 ± 0.10[d]	4.51 ± 0.20[c]
Plasma, ml/kg	51	50	45	45
Haematocrit (per cent)	42 ± 1	42 ± 2	41 ± 1	42 ± 1
Blood volume (ml)	18.20 ± 0.96	20.74 ± 0.65	18.84 ± 0.61	20.58 ± 1.40
Blood volume (per cent of body weight)	8.73 ± 0.17	8.75 ± 0.38	7.72 ± 0.15[d]	7.77 ± 0.32[c]
Blood volume (ml/kg)	90	86	77	78
Total body water (g)	140 ± 3.0	—	142.8 ± 3.7	180 ± 5.7
Total body water (per cent of body weight)	63.9 ± 0.84	—	63.3 ± 0.78	62.9 ± 1.9
Dry substance (g)	79.0 ± 3.0	—	82.7 ± 3.1	106.0 ± 6.2
Dry substance (per cent of body weight)	36.1 ± 0.93	—	36.7 ± 0.61	37.1 ± 1.6

a = $p < 0.05$; b = $p < 0.02$; c = $p < 0.01$; d = $p < 0.001$
* = body weight at the time of intervention 196 ± 9 g

4. Between the 30th and 37th week after the induction of hypertension, mean blood pressure remained permanently at 167 ± 8 mmHg. Plasma volume measured in 5 animals was 4.51 ± 0.2% and blood volume, computed with reference to the haematocrit value of 42%, was 7.77 ± 0.3% of body weight. The reduction in both values was significant statistically ($p < 0.01$). Total body water volume measured in 6 rats showed no deviation from the control values.

Discussion

There is ample experimental evidence to indicate that hypertension involves a disturbance of electrolyte and fluid metabolism, primarily reflected in an enhanced renal excretion of sodium and water (PICKERING, 1955; COTTIER et al., 1958; HANENSON et al., 1963; SELKURT et al., 1965; GORDON et al., 1967; LOWITZ et al., 1968; MIKSCHE, 1970; DICKINSON, 1971; FEKETE et al., 1970, 1971a). The sodium and fluid losses were attributed by ULLMANN et al. (1963) to a hypersensitivity of volume regulation of the hypertensive organism; by MARTINO and EARLEY (1967) to a direct effect of the increased perfusion pressure; by NICKERSON and SUTTER (1964) to a reduction of plasma volume due to angiotensin; by VANDER (1963) to a direct inhibition by angiotensin of tubular reabsorption; by DAVIS et al. (1966) to the "escape" phenomenon in connection with DOC; by RECTOR et al. (1968) to the production of a substance inhibiting tubular electrolyte absorption; by GORDON et al. (1967) to a reduction of plasma volume in consequence of enhanced sympathetic activity associated with chronic hypertension; by FEKETE et al. (1971b) to an enhanced renin

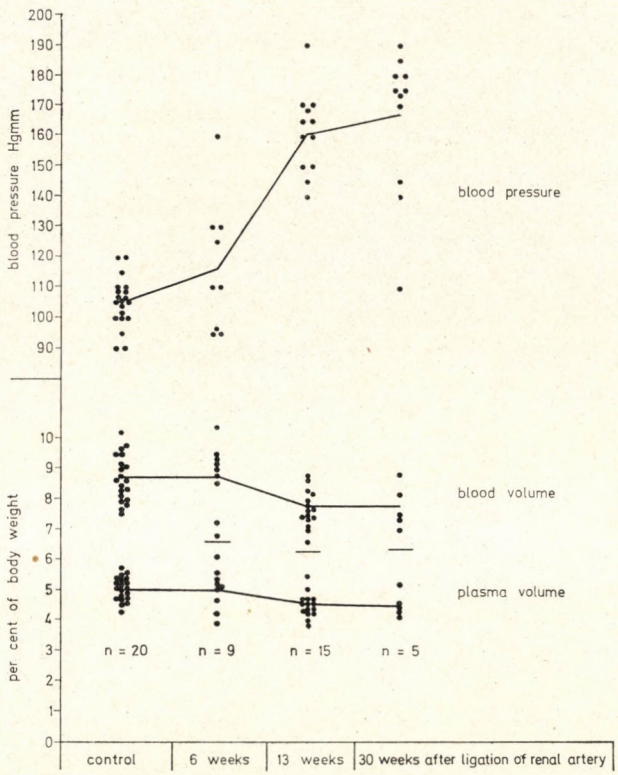

Fig. 1. Plasma, blood and total fluid volumes in rats at successive stages of experimental renal hypertension and in normotensive controls

activity demonstrable in the venous blood of the ischaemic and unmanipulated kidney alike; by THURAU (1962) as well as by GIRNDT and OCHWADT (1969) to a fall in the osmotic gradient due to the enhancement of medullary blood flow; and, finally, by MARTINEZ-MALDONADO (1971) to the vasodilating activity of prostaglandins.

In the present experiments the intravascular and total fluid compartments of rats with permanent hypertension have been studied at successive stages of the hypertensive condition.

The fluid compartments have been studied in human hypertensive disease, too. In this condition, ROCHLIN et al. (1959) found a 12% reduction in blood volume. Similar findings in respect of plasma volume have been reported by GORDON et al. (1967). GREEN and SAPIRSTEIN (1952) rejected the possibility of an expansion of the intravascular and extracellular compartments in hypertensive disease. TOBIAN (1960) found normal extracellular fluid compartments in the 3rd to 5th month of hypertensive disease. WALSER et al. (1956) as well as WINER (1957) examined all components of the fluid spaces without, however, finding any abnormality. According to GRAEFF (1957) the inulin-space was similar in normal and hypertensive subjects. ROSS (1956) found a 2% increase in total body water volume in hypertensive disease. In the studies of GREGERSEN et al. (1939) plasma volume as estimated with T-1824 was 50 ml/kg in hypertensive subjects. TARAZI (1967), on the evidence of radioisotope studies, found plasma volumes of 42.3 ml/kg in hypertensive disease as against 48 ml/kg in normotensive subjects. The findings of CONWAY (1967) connected the early stage of renal hypertension with an expansion of the extracellular fluid compartments. In opposition to this, BIANCHI (1970) found an unaltered plasma volume in hypertension.

As regards induced renal hypertension in animal experiments, LEDINGHAM (1964) found in dogs a transitory increase in EC volume including that of plasma, between the 7th and 14th day, normalization ensuing after the 14th day. In a dog with Goldblatt type hypertension (induced by renal arterial constriction (GROLLMAN and SHAPIRO, 1953) found an increased extracellular volume. In earlier studies of the present authors (FEKETE et al., 1971c) a reduction of the intra and extracellular compartments was noted between the 8th and 9th months of the chronic phase of experimental renovascular hypertension in dogs. For the dog's normal plasma volume GREGERSEN and STEWART (1939) found 48 to 64 ml/kg, COURTICE (1943) 56 ml/kg, FEKETE et al. (1971c) 60 ml/kg.

On the evidence of the data concerning Wistar rats in DONALDSON's classical monograph (1924) after the time of sexual maturity i.e. the age of two months, blood volume increases parallel with body weight, in other words, blood volume expressed in per cent of body weight remains unchanged. The total fluid volume corresponds to approximately 67% of body weight. Accord-

ing to CASTER et al. (1955) the plasma volume yielded by the Evans blue method, corresponds to 4.8% of body weight in the normal rat.

Rats with Goldblatt-type hypertension being in possession of both kidneys (one kidney with constricted renal artery, the other kidney unmanipulated) revealed normal EC compartments in the 3rd to 5th month of hypertension (TOBIAN, 1960). LEDINGHAM (1964) found a rise in plasma volume in hypertensive rats with a single kidney during the first 40 days of hypertension. Subsequently, normalization of plasma volume ensued parallel with the stabilization of hypertension. BRAUN-MENENDEZ and MARTINEZ (1949) noted an expansion of the intravascular and extracellular compartments 3 to 5 months after the production of hypertension. KRAMER and OCHWADT (1971) demonstrated a slight increase in total body water one week after unilateral nephrectomy, at a time when blood pressure was still within normal limits. (Normal value, 64.6%, after nephrectomy, 66.7%, of body weight).

In the present study, 6 to 8 weeks after unilateral ligation of the renal artery, in the presence of an unmanipulated opposite kidney, blood pressure and the fluid compartments were practically the same as in the controls. Thirteen weeks after the intervention, a significant elevation of blood pressure was found together with a significant reduction in plasma and blood volume, while total fluid volume was unaltered. Between the 30th and 37th week after the intervention there was likewise a significant elevation of blood pressure and a significant reduction in plasma and blood volume, while total body water was unaffected. The values found with a normal blood pressure were 51 ml/kg for plasma volume, 90 ml/kg for blood volume, and 63.9% of body weight for total body water. At blood pressures of approximately 170 mmHg, plasma volume was 45 ml/kg, blood volume 78 ml/kg (haematocrit being unaltered), and total body water, 63.1% of body weight. The serum sodium level was 150 mEq/L in the normotensive and hypertensive animals alike.

The reduced plasma and blood volume in chronic experimental renal hypertension would seem to indicate that the regulatory mechanisms of renal origin affecting the fluid compartments have suffered certain alterations which, however, have yet to be clarified.

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EFFECT OF CYTOSTATIC AGENTS ON THE ADHESION OF HUMAN AMNIOTIC CELL LINES

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A rapid and simple in-vitro screening test for cytostatic agents is described. By means of ^{51}Cr-labelled cells, it measures the loss of adhesivity of amniotic cells resulting from the cytostatic action of these agents. The possible relationships between inhibition of cell adhesion and suppression of metastatic implantation are discussed.

It has been demonstrated by EAGLE and FOLEY [2—4] that cell cultures provide a screening tool for the action of cytostatic agents. Apart from certain disadvantages, the procedures making use of cell cultures have numerous advantages over animal experiments and have found general acceptance.

Isotope release of ^{51}Cr-labelled cells (Chang, amnion) has been widely used for the detection of immunological cytotoxic reactions [1, 6, 7, 9]. On the evidence of earlier studies [8], inhibition of the adhesion of ^{51}Cr-labelled cell monolayers constitutes a more sensitive procedure for the demonstration of cytotoxic reactions than does isotope release. It seemed therefore of interest to examine the inhibitory action of cytostatic agents on the adhesion of cell monolayers.

Material and methods

Human amniotic cells were obtained from the Institute of Microbiology, Debrecen. The cells after labelling with ^{51}Cr were washed repeatedly with the TC-199 medium and made up to a concentration of 10^5/ml with the medium containing 10% calf serum. Then 1 ml of the cell suspension and 0.1 ml of the diluted cytostatic agent were measured into sterile 10 ml tubes and made up to 1.5 ml with the medium. The tubes were stoppered and incubated in a slanted position at 37°C for 24 hours. Then the nutrient medium was poured off, the tubes were rinsed repeatedly with isotonic saline and the radioactivity of the cells adhering to the wall of the tubes was measured in parallel samples. The activities thus obtained were expressed in per cents of the values found in the controls.

For the demonstration of the cytostatic action of the agents, supravital trypan blue staining was used, a 0.25% solution of trypan blue being added to the supernatant. The viable cells were then counted in a Buerker-chamber.

ED_{50}, i.e. the dose inhibiting the adhesion of 50% of the cells, was computed on the basis of the BEHRENS—KÄRBER formula [5].

Results

The action of the cytostatic agents on the adhesion and viability of the cells is shown in Table I. All of the agents under study revealed a greater potency, i.e. lower ED_{50} figures, in this test than on the grounds of supravital

staining (cytotoxicity). The ED_{50} values yielded by the two procedures gave a five- to tenfold difference. In fact, the amniotic cells proved fairly resistant to cytostatic agents, particularly to metothrexate. The detergent (Tween 80) used for reference purposes has been found definitely to inhibit adhesion at

Table I

Effect of cytostatic agents on the adhesion and viability of human amniotic cells

	ED_{50} concentration inhibitory to adhesion μg/ml	ED_{50} concentration cytotoxic μg/ml
Mannomustine	11.3	50—100
R-74 (dimesylerythritol)	9.7	10—25
Dibromodulcitol	250	250—500
Hydroxyurea	3300	>5000
Vinblastine	23	25—50
Rubidomycin	0.9	5—10
Metothrexate	>1000	>1000
Tween 80	8	>1000

concentrations as low as 50 μg/ml which is still below the cytotoxic level. The ED_{50} obtained by the two procedures for Tween 80 showed a significant difference.

The observation that at higher concentrations some of the cytostatic agents shift the pH of the medium in the direction of acidity by exhausting its buffer capacity has made us to test the influence of pH on cell adhesion. A decrease of the pH below 5.5 resulted in an abrupt fall in the number of adhering cells to a few per cent. On the other hand, above pH 5.5, cell adhesion amounted to nearly 100%.

Discussion

The procedure based on the ratio of adhering and nonadhering cells allowed to assess the extent of functional inhibition. We found this test to represent a more sensitive indicator of cytotoxic activity than for instance the dye exclusion method. The inhibitory effect of cytostatics on the adhesion of monocellular layers is believed to result from a direct but very slight cytotoxic activity operating below the cytocide level. Inhibition of cell adhesion, viewed as a method, examines the alterations of the cell surface. Cytostatics of the alkylating type, being apt to produce a direct lesion to the cell membrane, might be expected to inhibit the adhesion of monocellular layers even in sublethal doses, as opposed to nonalkylating agents which would seem to require

massive doses for affecting cell adhesion. Experimental evidence has, however, failed to bear out this conjecture. In fact, no difference in inhibitory activity has been found between alkylating or nonalkylating agents.

The pH-changes induced by the cytostatics had no influence on cell adhesion. At the concentrations applied in the present study, the pH was above 6.0 with all the cytostatics examined, a pH at which cell adhesion is unaffected.

The adhesion inhibition test lends itself to the study of the surface activity of noncytostatic substances as well. On exposure to nontoxic concentrations of Tween 80, the monolayers fail to adhere to the glass surface. Would the cytostatic agents examined exert their inhibitory action on cell adhesion by primarily affecting the cell membrane, then the difference between the two ED_{50}-values would have to be as great as in the case of Tween 80.

It is inferred from these results that the inhibition of adhesion is due to the cytostatic properties specific of the given agent and the reason why it indicates more sensitively the toxic effect than other current tests is because long before its total destruction the cell loses its capacity of adhesion in consequence of alterations produced on its surface.

It would be reasonable to predict an antimetastatic activity in the first place for those agents which affect the cell membrane, in other words, which have an inhibitory effect on cell adhesion. Further studies are planned in order to test the possible correlations between the inhibitory effect of cytostatics drugs on cell adhesion and their preventive effect on metastatic implantation in laboratory animals.

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IMMUNOGLOBULINS, GLYCOPROTEIDS AND AUSTRALIA ANTIGEN IN CHRONIC LIVER DISEASE

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Sera of patients with chronic liver disease have been studied for the concentrations of IgG, IgA, IgM, coeruloplasmin, α-2-macroglobulin, β-1-C globulin and transferrin, by the radial immunodiffusion procedure, furthermore for the titres of humoral antibodies reacting with nuclear, mitochondrial and soluble protein antigens from allogenic liver by the passive haemagglutination method, finally for Australia antigen and for its antibody-titre by a microcomplement fixation procedure. In chronic persistent hepatitis the IgA and IgM levels, in chronic aggressive hepatitis and in cirrhosis of the liver those of all three immunoglobulins showed a significant increase as compared with the controls. In chronic aggressive hepatitis, the increase in IgG was particularly marked. The serum coeruloplasmin and α-2-macroglobulin levels were increased in relation to the extent of the inflammatory process involving the hepatic stroma. In contrast, β-1-C globulin and transferrin were not significantly affected. Australia antigen and humoral antibodies were encountered most frequently in chronic aggressive hepatitis.

The aetiology of chronic hepatitis is still unclear, in spite of extensive research on the subject. Inflammation and hepatocellular lesion may be due to various factors such as infectious mononucleosis, Australia antigen (Au/SH antigen), alcohol, drugs or other agents. As tissue lesions in general, the primary hepatocellular lesion is also accompanied by a mesenchymal reaction, consisting in a proliferation of fibroblasts, macrophages, lymphocytes and plasma cells. The activity of these cells result in fibrosis with the appearance of sessile and humoral antibodies, formation of antigen-antibody complexes and, finally, in a secondary hepatocellular lesion, a vitious circle being thus generated.

We have studied the immunological aspects of chronic hepatitis and cirrhosis of the liver in a clinical material, with especial attention to the possible relationships between the intensity of mesenchymal reaction characteristic of the inflammatory process affecting the hepatic substrate and the serum glycoproteid levels.

Material and method

Serum of hospitalized patients was used in the studies. Chronic hepatic disease was diagnosed on the basis of the history, clinical features, laboratory tests, and histological and histochemical findings of liver biopsy material. Liver biopsy was performed if of the tests for SGOT, SGPT, LDH, BSP, colloid lability, serum bilirubin level, prothrombin time, IgG at least three revealed abnormal values. Classification of the cases into individual groups was based on morphological evidence. The data of 91 patients selected on the basis of histological

diagnosis from approximately 300 cases have been utilized. The patient material consisted of 16 cases of chronic persistent hepatitis, 25 cases of chronic aggressive hepatitis, 30 cases of cirrhosis of the liver and 20 cases of fatty infiltration. Cases of cirrhosis exhibiting tissue activity were only considered.

For the estimation of IgG, IgA, IgM and coeruloplasmin, α-2-macroglobulin, β-1-C-globulin and transferrin, the radial diffusion technique described by MANCINI et al. [19] was used. Pooled serum derived from 100 blood donors served as control. Values expressed in percentage were translated into absolute values on the basis of reference sera (HYLAND, KALLESTAD), and in the case of transferrin and β-1-C-globulin, by correlation with the data of BECKER et al. [5]. Results were evaluated statistically by Student's *t* test.

For the demonstration of Au/SH antigen and antibody, the microcomplement fixation procedure [35] was used. The titres of humoral antibodies against allogenic hepatocellular nucleus, mitochondria and soluble protein were estimated by passive haemagglutination according to the procedure described by SZÉCSEY et al. [34].

Results

The proportion of Au/SH antigen-positive sera was 16 : 3 in chronic persistent hepatitis, 25 : 8 in chronic aggressive hepatitis and 30 : 2 in cirrhosis of the liver. No positive reaction was found in any of the cases of steatosis of the liver. Anti-Au/SH antigen antibodies were found in 8 cases. In the absence of Au/SH antigen, the presence of anti-Au/SH antibody was confined to one patient with chronic aggressive hepatitis.

The sera revealing identical antibody and self-binding titres were assigned to the Au/SH antibody-negative group. Self binding, suggestive of circulating soluble antigen-antibody complexes [31] was found in 7 cases of chronic aggressive hepatitis, in 2 of chronic persistent hepatitis and in one of cirrhosis of the liver. In the cases of hepatic steatosis the self-binding reaction was negative all throughout.

The humoral antibody titres were measured against soluble protein, mitochondrial and nuclear antigens. Distribution of antibody titres is presented

Table I

Humoral antibody titres in chronic liver disease

Antibody titre	Chronic persistent hepatitis			Chronic aggressive hepatitis			Cirrhosis of liver			Steatosis of liver		
	S	M	N	S	M	N	S	M	N	S	M	N
Negative	14	13	13	22	20	21	28	28	28	18	18	18
Positive	2	3	3	3	5	4	2	2	2	2	2	2
1 : 8	2	2	2	1	2	0	1	0	0	1	1	1
1 : 16	0	1	1	0	1	2	1	1	0	1	1	1
1 : 32	0	0	0	1	0	1	0	1	1	0	0	0
1 : 64	0	0	0	1	1	0	0	0	1	0	0	0
1 : 128	0	0	0	0	1	1	0	0	0	0	0	0

S = soluble protein; M = mitochondrium; N = nucleus.

Table II

Immunoglobulin- and glycoproteid concentrations (mg/100 ml) in chronic liver disease

		Normal	Chronic persistent hepatitis	Chronic aggressive hepatitis	Cirrhosis of liver	Steatosis of liver
IgG	*n*	25	16	25	30	30
	x	1200	1525	2320	1810	1396
	s	280	175	321	271	169
	p		>0.05	<0.001	<0.05	>0.05
IgA	*n*	25	16	25	30	20
	x	210	334	363	568	289
	s	80	51	47	116	47
	p		<0.05	<0.01	<0.01	>0.05
IgM	*n*	25	16	25	30	20
	x	130	265	184	242	200
	s	53	47	32	42	37
	p		<0.01	<0.01	<0.01	>0.05
Coeruloplasmin	*n*	25	16	25	30	20
	x	31	49	53	54	53
	s	11	7	6	9	11
	p		<0.05	<0.01	<0.05	<0.05
Alpha-2-macroglobulin	*n*	25	16	25	30	20
	x	244	283	367	368	272
	s	60	26	42	55	34
	p		>0.05	<0.01	<0.05	>0.05
Beta-1-C-globulin	*n*	25	16	25	30	20
	x	110*	128	118	108	154
	s	20	12	6	4	26
	p		>0.05	>0.05	>0.05	>0.05
Transferrin	*n*	25	16	25	30	20
	x	295*	334	345	361	326
	s	55	28	49	39	32
	p		>0.05	>0.05	>0.05	>0.05

n = number of cases; x = mean; s = standard deviation; p = significance; * = according to BECKER et al. [5].

in Table I. Antibodies reacting with allogenic antigens occurred mainly in chronic hepatitis.

There was a considerable elevation of the IgG level in chronic aggressive hepatitis and in cirrhosis of the liver. The IgA level increased in both types of

chronic hepatitis as well is in cirrhosis of the liver. This was also true for IgM, the highest concentrations of which were found in chronic persistent hepatitis. In steatosis of the liver, no appreciable changes were observed (Table II).

As regards the serum glycoproteids, a significant elevation of the coeruloplasmin level was demonstrable, in chronic hepatitis, cirrhosis of the liver, and also in steatosis of the liver (Table II), with the highest mathematical significance in chronic aggressive hepatitis. The α-2-macroglobulin underwent a significant increase in chronic aggressive hepatitis and in cirrhosis while in chronic persistent hepatitis and in steatosis of the liver its changes were not significant. No appreciable changes in the β-1-C-globulin and transferrin levels occurred in any of the groups.

Discussion

The two prevailing theories accounting for the pathogenesis of chronic liver disease, the first claiming a viral, the second an autoimmune origin, are based on weighty evidence. The claim of viral origin is supported by the discovery of Au/SH antigen [6] and by its subsequent demonstration in the serum of numerous patients with chronic hepatitis and cirrhosis of the liver [1, 20, 30, 41]. On the basis of morphological, serological, epidemiological and physicochemical criteria, the Au/SH antigen seems to be identical with the virus of serum hepatitis or with some of its structural constituents [8]. WRIGHT [39] found Au/SH antigen in 14%, WEWALKA et al. [38] in 62% of sera in chronic active (aggressive) hepatitis. PRINCE [26] reported a figure of 5.5%, SANWALD et al. [27] of 23% for the positive sera in cirrhosis of the liver. The recent findings of NIELSEN et al. [22] indicate that patients with acute hepatitis in whom the Au/SH antigen persists, are liable to develop chronic hepatitis. Hungarian literature also contains ample evidence on this subject [2, 13, 16, 17, 21]. SZÉCSEY et al. [34] have shown the presence of Au/SH antigen in acute serum hepatitis in 78%, in chronic hepatitis in 60% and in cirrhosis of the liver in 40%. In the present patient material, the proportion of Au/SH positive cases was smaller: the figures of Au/SH positivity were 18% for persistent hepatitis, 32% for aggressive hepatitis and 6% for cirrhosis of the liver. This may be due to the fact that our cases have been derived from an unselected material admitted for various causes, moreover, a number of our patients had had acute hepatitis many years earlier. On the other hand, the material of SZÉCSEY et al. [34] consisted of patients regularly followed up after acute hepatitis.

The autoimmune origin of chronic liver disease is substantiated by the presence of various antibodies in the sera of these patients. Anti-smooth-muscle antibodies have first been described in lupoid hepatitis [18] but on the evidence of more recent research, they are found in other forms of acute

hepatitis too, and often also in chronic hepatitis [7, 37, 40]. The antinuclear antibodies form a further group. No antibodies of this type have been found in acute viral hepatitis or in drug-induced jaundice. On the other hand, their presence is fairly typical of lupoid hepatitis and they may occur in chronic aggressive hepatitis, in cryptogenic cirrhosis and in primary biliary cirrhosis [7, 10, 23, 33]. The third main group includes the mitochondrial antibodies which, according to DONIACH [11], are demonstrable in 90% of the cases of primary biliary cirrhosis while being practically always absent in biliary obstruction, pericholangitis and viral hepatitis, a finding of diagnostic implications. BALÁZS et al. [3] studying the immune responsiveness by means of the lymphocyte transormation test noted an impairment of the response in autoimmune liver disease together with the development of delayed type hypersensitivity to hepatocellular mitochondrial material. Humoral antibodies directed against subcellular liver fractions are known to be present in numerous cases of chronic hepatitis [4, 12, 33]. In the present observations, antibodies against hepatocellular nuclei, mitochondria and soluble protein occurred in chronic persistent hepatitis in 18%, in chronic aggressive hepatitis in 20%, in cirrhosis of the liver in 6% and in hepatic steatosis in 10%. These results are comparable to the recent observations of SZÉCSEY et al. [34] on a different patient material. There is also earlier evidence indicating that immunological manifestations are distinctive of certain types of chronic inflammatory liver disease [9, 24]. Abnormal immune reactions and protein changes have been demonstrated in chronic hepatitis, in the first place in its aggressive and necrotizing types [25, 28, 32]. According to SCHUMACHER and GROSS [29], in chronic aggressive hepatitis the serum level of IgG, in chronic persistent hepatitis that of IgM is increased. These findings agree with our earlier observations [14]. In the present patient material an increase in the IgA and IgM levels was prevalent in chronic persistent hepatitis whereas chronic aggressive hepatitis and cirrhosis of the liver were associated with an increase in all the three immunoglobulin components. The maximum rise was found in cirrhosis of the liver, and the immunoglobulin component attaining the highest level was IgG.

The serum coeruloplasmin level was increased in chronic hepatitis as well as in cirrhosis and steatosis of the liver. An increase related to the intensity of the inflammatory change in the liver was demonstrable in the α-2-macroglobulin level. Its elevation was significant in the active, aggressive form of chronic hepatitis and in cirrhosis of the liver, and not significant statistically in chronic persistent hepatitis and in steatosis of the liver. No significant changes have been found in the serum transferrin and β-1-C-globulin levels, in agreement with our earlier observations [15].

In steatosis of the liver where, apart from a very slight lymphocytic infiltration of the portobiliary connective tissue, the biopsy revealed no sign of

an inflammatory reaction, no appreciable changes were noted in the levels of the immunoglobulins and of the individual glycoproteids, with the only exception of coeruloplasmin. The increase in the coeruloplasmin level may possibly reflect the inflammatory reaction of the liver, despite its minimum intensity, an interpretation in agreement with the observations of URBASZEK [36].

In agreement with data in the literature, the present results thus seem to suggest the existence of definite relationship between the persistence of Au/SH-antigen and the production of chronic parenchymal injury in the liver. Moreover, they support the claim that the immune system is affected by chronic hepatitis. The increase in the α-2-macroglobulin and coeruloplasmin levels, reflects in our view the intensity of the inflammatory reaction of the hepatic stroma.

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SECRETAGOGUE GLOBULINS IN THE BLOOD OF DUODENAL ULCER PATIENTS

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The cause of hypersecretion exceeding the usual regulation mechanisms in duodenal ulcer patients is unclear. In the present study, in 7 out of 17 hyperchlorhydric subjects with duodenal ulcer the blood globulin fraction has been found to elicit a significant gastric hypersecretion in rats. It is suggested that in certain cases the presence in blood of a secretagogue factor of the immunoglobulin type or an autoimmune process might be responsible for the production of gastric hypersecretion.

The aetiology of peptic ulcer has not been clarified but for the different types of ulcer different pathogenetic factors seem to be responsible [1—3]. Duodenal ulcers are often marked by a substantial gastric hypersecretion in comparison with the normacid general population. It is remarkable that no connection could be established between this type of hypersecretion and the hyperactivity of the physiological regulatory mechanisms of gastric secretion [4—8]. On the evidence of histological and other studies, in duodenal ulcer patients, the HCl-producing parietal cells have been found to increase their mass to the extent of superseding the physiological secretory phases (cephalic, gastric, enteral). As a result, at least in the periods of activity, they are continuously secreting, giving thus rise to an excessive basal secretion even in the resting state of the stomach [9—12]. It may be questioned whether it is actually gastrin alone which accounts for the figures of "gastrin-like" activity yielded by biological titrations for gastrin-activity of sera or of serum concentrates [13, 14].

Pernicious anaemia is known to be due to an autoimmune mechanism resulting in gastric achlorhydria [15]. Conversely, there are immunoglobulins of secretagogue properties, as exemplified by Lats [16—19]. It has been assumed on these grounds that autologous immunoglobulins may be responsible for hyperacidity associated with duodenal ulcer. It has been sought to clarify this issue by examining the serum globulins of duodenal ulcer patients for secretagogue activity by means of biological titration. Hyperchlorhydria of the patients was determined by testing their gastric secretion under basal conditions and after augmented histamine test.

Material and method

In anaesthetized rats, after continuous slow gastric lavage for 1 hour the gastric juice was titrated for free HCl, then, after administration of serum globulin obtained from healthy control subjects and of duodenal ulcer patients, gastric secretion was studied by repeated titration over 6, 8 and 10 hours. For the separation of globulin, the serum was dialyzed in phosphate buffer pH 5.8 for 48 hours, washed three times with distilled water for the removal of albumin and of possible polypeptide contaminations and the precipitate was dissolved in TRIS buffer of pH 8. The technique precluded the presence of gastrin or of other known secretion-stimulating factors in the fraction. Twentyfive tests with globulin obtained from 17 ulcer patients were carried out, against 11 control tests.

Table I

Production of HCl/hour by rat stomachs, μg

C hour	111	34	58	467	624	416	88	817	108	286	703
1.0 ml i. v. →	ø	0.9 NaCl	TRIS buffer	TRIS buffer	m. g. B. B.	m. g. B. B.	TRIS buffer	ø	m. g. (21y)	m. g. (21y)	m. IgG (21y)
1. hour	126	43	57	305	189	146	71	307	95	198	304
2. hour	131	57	73	350	62	88	54	277	108	216	166
3. hour	131	43	88	350	84	114	69	188	117	202	131
4. hour	117	56	88	365	83	102	28	162	148	207	135
5. hour	120	53	85	336		208	28	81	83	97	74
6. hour	135	42		291			41	114	87	57	74
7. hour		42		394				97	83	57	69
8. hour									83		

Maximum increase: 1.6 fold; maximum decrease: 0.1 fold.

Results

Table I shows the results of control tests, which have been undertaken for the verification of the possible secretory changes expressed in HCl/hour which may occur spontaneously or in response to non-specific interventions such as the intravenous administration of physiological saline, of TRIS buffer or of globulin derived from pooled sera of healthy subjects, in experiments continued for 8 to 9 hours. As compared to the basal figures set out in the uppermost row *C*, the maximum increase was 1.6 fold and the maximum decrease 0.1 fold. This is represented also in Fig. 1 where, instead of giving absolute figures, the acidity values of the successive hours have been referred to the basal values. The first small column represents the basal level (row *C* in Table I), regardless of the differences in HCl/hour secretion in the individual animals. As referred to these values, the majority of subsequent ones showed a decrease.

The results of these experiments are shown in Table II, in the same arrangement as in Table I and in Fig. 2. Ten out of 25 measurements revealed

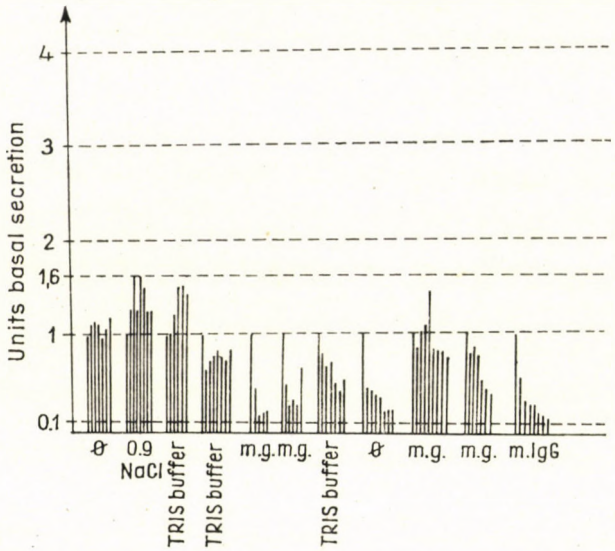

Fig. 1. Production of HCl/hour by rat stomach as referred to basal secretion. Vertical axis: units of basal secretion; horizontal axis: groups and symbols as in Table I

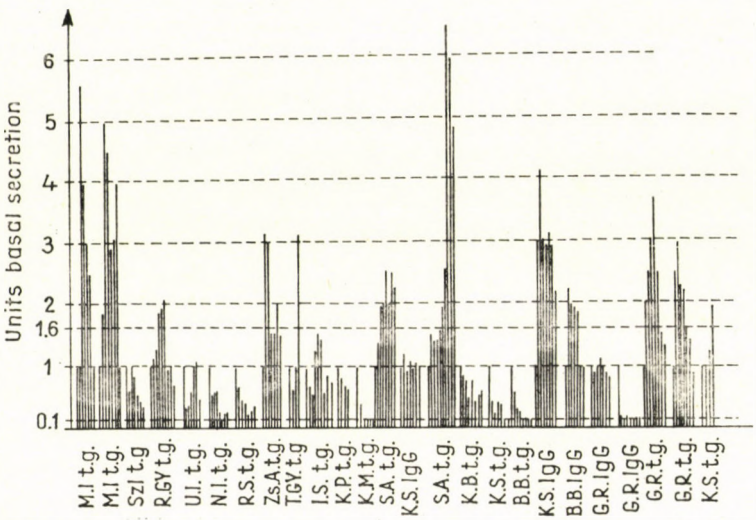

Fig. 2. Production of HCl/hour by rat stomach in response to globulin obtained from subjects with hyperchlorhydric, as referred to basal secretion. Vertical axis: units of basal secretion; horizontal axis: groups and symbols as in Table II

a more than twofold increase in HCl-secretion. These increased values were caused by the globulin of 7 patients; the globulin of 3 of these (M. I., S. A., K. S.) caused a fourfold increase by the end of the 3rd, 4th and 8th hour. Repeated tests yielded identical results in three cases (M. I. in columns 1—2, S. A. in columns 13—15 and G. R. in columns 23—24).

Table II

Acid secretion in HCl/hour by rat stomachs after intravenous

C hour	137	39	418	183	139	504	172	29	46	307	459
1.0 ml → i. v.	M. I. t. g.	M. I. t. g.	Sz. I. t. g.	R. Gy. t. g.	V. I. t. g.	N. J. t. g.	R. S. t. g.	Zs. A. t. g.	T. Gy. t. g.	I. S. t. g.	K. P. t. g.
1. hour	110	73	222	201	28	189	116	88	29	208	321
2. hour	766	790	414	219	55	184	88	29	44	160	277
3. hour	610	175	307	328	81	194	88	88	146	360	256
4. hour	402	107	195	347	0	83	73	43		456	
5. hour	323	120	134	365	118	55	29	42		429	
6. hour	146	147	131	168	166	139	43	58		102	
7. hour		29		168	59	142	73	44		284	
8. hour				122						234	
9. hour											
10. hour											
11. hour											
12. hour											

In the second line, initials of the patients from whom the globulins have been obtained.

Discussion

It is safe to assume that, similarly to the well-established mechanisms involved in gastric secretion, also those which have yet to be explored, in particular the immune regulations, may be affected in some manner. It is well-known that under certain conditions the immune system may play a significant part in the production of hypertrophy and hyperplasia [20—23]. The gastro-intestinal involvements due to immunological disturbances present a wide diversity, from an acute abdomen [24—26] to pernicious anaemia, chronic autoimmune gastritis, and even certain types of malabsorption [27—30].

Though human peptic ulcer is a kind of fibrinoid necrosis similar to those associated with collagen or autoimmune disease, ulcers have been regarded as nonspecific [31].

The present results would seem to suggest that hypersecretion associated with duodenal ulcer might be due in certain cases to a globulin type stimulating factor. Further studies are in progress to identify and isolate the factor carrying the stimulating activity.

administration of globulin obtained from patients with hyperchlorhydria

1338	42	92	58	125	759	1610	41	87	125	708	105	131	214
K. M. IgG	S. A. t. g.	K. S. IgG	S. A. t. g.	K. B. IgG	K. S. t. g.	B. B. t. g.	K. S. IgG	G. R. IgG	G. R. IgG	G. R. IgG	G. R. t. g.	G. R. t. g.	V. S. t. g.
321	57	122	84	108	235	805	43	156	125	160	226	199	222
180	83	88	78	92	162	362	128	190	110	69	263	308	277
100	85	83	77	55	207	312	152	186	131	57	328	393	407
85	108	105	84	99	214	236	139	140	134	73	312	284	
139	85	95	88	41	114	270	131	147	125	74	366	278	
114	102	97	105	41	99	176	111	85	143	57	255	234	
	95	97	148	41		194	131	83	117	57	156	184	
			408	54		143	117	87	102	74	139	128	
			384			161	99						
			307										
			152										

IgG = IgG solution; tg = total globulin.

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5-HYDROXYINDOLEACETIC ACID IN CEREBROSPINAL FLUID

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The concentration of 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid has been estimated in 95 neuropsychiatric cases. The level was found to vary according to age and to present a wide scatter. The values tended to be high in juveniles as well as in subjects of advanced age, while lower in middle aged ones, but the differences were not significant statistically. The lowest concentration of 5-HIAA in CSF (8.1 ± 4.5 ng/ml) was found in the group of depressive disease. In epileptics and patients with organic cerebral disease the 5-HIAA level was definitely higher than in the previous group (15.4 ± 5.0 and 15.3 ± 7.0 ng/ml, respectively). Lower concentrations were found in the drug-free group, but the difference was not significant. The level was extremely low (5 to 8 ng/ml) in two subjects with grave brain-stem lesion, in one with compression of the spinal cord and in one with taboparesis.

The effect on the CSF 5-HIAA level of tryptophan and probenecid loading has been studied in view of eventual diagnostic possibilities.

The possibilities of studying the turnover of biogenic amines in patients are naturally restricted, but the present range of diagnostic investigations of the CSF currently applied in neuropsychiatry can still be extended. Recent techniques allow to estimate the serotonin metabolites in 1 ml CSF, an amount largely covered by the usual samples collected for routine tests. In other words, the procedure requires no additional lumbar puncture thus avoiding unnecessary discomfort to the patient.

It has been questioned whether the levels of biogenic amines or of their metabolites in the CSF are likely to furnish any useful information, it being uncertain whether the various factors affecting the CSF constituents bear any relationship to cerebral metabolism.

It has been confirmed experimentally that the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) does not pass across the blood-brain-barrier (ROOS, 1962; MOIR and ECCLESTON, 1968). The findings of GULDBERG and YATES (1968), ANDERSON and ROOS (1968) and others indicate that the CSF 5-HIAA level reflects the cerebral turnover of serotonin. The 5-HIAA formed in cerebral tissue passes into the CSF, and a state of equilibrium is established between the brain and CSF 5-HIAA levels. It is true that certain factors provide a source of misinterpretations in respect of serotonin turnover, in particular the elimination from the CSF of the acid metabolite, the rate of its passage into the blood, the activity of the transport process (GULDBERG and YATES, 1968). Allowance being made for these factors, measurement of metab-

olites in the CSF offers an informative angle of approach to the metabolism of the brain (MOIR et al., 1970).

In human pathological conditions, additional factors may affect the CSF 5-HIAA level. The relationships between certain syndromes and the 5-HIAA level have been widely studied. On the evidence of recent observations, the 5-HIAA concentration in the CSF is definitely age-dependent, as has been shown in healthy volunteers, too (GOTTFRIES et al., 1971). Drugs also affect the 5-HIAA level, it is, however, difficult to establish, how far the changes are due to some interferences or to the actual drugs.

In the laboratory animal, the CSF 5-HIAA level was shown to increase in response to reserpine and to remain unaffected by imipramine (ASHCROFT and SHARMAN, 1962). Chlorpromazine produced an increase in the concentration of acid metabolites in the caudate nucleus of the dog, parallel with a rise in the metabolite levels in the CSF (MOIR, 1967). Under tryptophan loading the cerebral serotonin concentration increased (MOIR and ECCLESTON, 1968) while intravenous administration of α-methyl-dopa and of triptophan was followed by an elevation of the 5-HIAA concentration in the brain-stem, as reflected by a parallel rise in the 5-HIAA level in the cisternal fluid (ECCLESTON et al., 1962). 5-hydroxytriptophan was found to raise the concentration of 5-HIAA in the CSF in parkinsonism (CHASE et al., 1962). An elevation of the HIAA, in addition to those and dopamine levels, was produced by l-dihydroxyphenylalanine (l-dopa). An increase in the cerebral concentrations of 5-HIAA was demonstrable in autopsy material of subjects with Parkinson's disease having been treated with l-dopa (RINNE, 1971). On the other hand, the CSF 5-HIAA level was found to decrease in the course of long-term administration of l-dopa (WOERT et al., 1970). On the evidence of the studies of NEFF et al. (1964), GULDBERG et al. (1966), ASHCROFT et al. (1960) and of others, probenecid inhibits the transport of acid metabolites from the CSF to the blood stream. This accounts for the probenecid-induced excessive rise in the concentrations of homovanillic acid (HVA) and of 5-HIAA in the CSF.

We have measured the concentration of 5-HIAA in the CSF in neuropsychiatric cases, with the aim of collecting information concerning the factors affecting that level. We have analyzed the age relationships of the changes, furthermore, the influence of drugs by setting up a separate untreated group and compared the results with those obtained in epileptics and depressive cases. Moreover, the response of the CSF-5-HIAA level to loading with tryptophan and with probenecid have been studied.

Material and methods

A total of 102 CSF samples obtained from 95 patients were studied for the concentration of 5-HIAA. Estimation of 5-HIAA was done by the modified spectrophotofluorimetric technique of KORF et al. (1969). CSF (cisternal in 12, lumbar in 83 cases) was stabilized with 4%

cystine and stored at —20°C before processing. Then it was shaken with diethyl ether and transferred from the etheric phase to a pH 7 phosphate buffer. After oxidation with periodate, fluorophore was formed with o-phthalaldehyde. The values were read at 365/495 nm (Opton) against standards. In addition to requiring no more than 1 ml CSF, the procedure has the advantage of being highly sensitive. (5 ng/ml corresponds to 80 divisions at a 0.5/0.5 slit and at a sensitivity of 3/3.) The concentration of HIAA was expressed in terms of ng/ml CSF. The deviations of the means were calculated by the *F*- and *t*-tests by paired comparison.

Results

The patients were divided into 4 age groups. The means and standard deviations were computed for each group. As Table I shows, the highest CSF 5-HIAA levels were found in the age-group below 20 years (15.2 $\pm$ 6.6). In the two subsequent groups the level was lower (12.1 $\pm$ 6.2 and 13.1 $\pm$ 7.5). Beyond the 6th decade the level again tended to increase (13.8 $\pm$ 6.8), but the differences were not significant statistically (Fig. 1).

Table I

Age and 5-HIAA level in CSF (ng/ml CSF)

Age group		20	20—40	41—60	over 60
5-HIAA	range of variation	8—30	4—25	5—31	5—30
	mean + S. D.	15.2 $\pm$ 6.6	12.0 $\pm$ 6.2	13.1 $\pm$ 7.5	13.8 $\pm$ 6.8
	number of patients	10	27*	40	17
	mean age	(13.7)	(33.3)	(49.3)	(70.9)

* Results of probenecid-loading performed in one case (130 ng/ml) have not been included in these figures.

The differences between the means for the individual age-groups were not significant statistically.

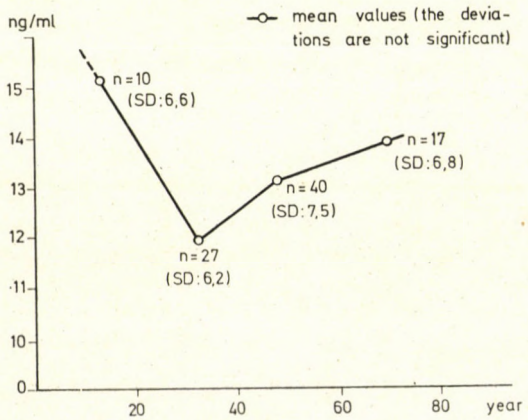

Fig. 1. Age and HIAA level in CSF

The groups were divided into six subgroups (depressive disease, other psychotic syndromes, epilepsy, organic cerebral lesion, other neuro-psychiatric diseases, drug-free group).

The means and standard deviations were computed for each group (Table II). Analysis of the data yielded the lowest 5-HIAA concentration in the group of depressive disease (8.1 ± 4.5), the highest in the group of epilepsy (15.4 ± 5.0, $p < 0.05$).

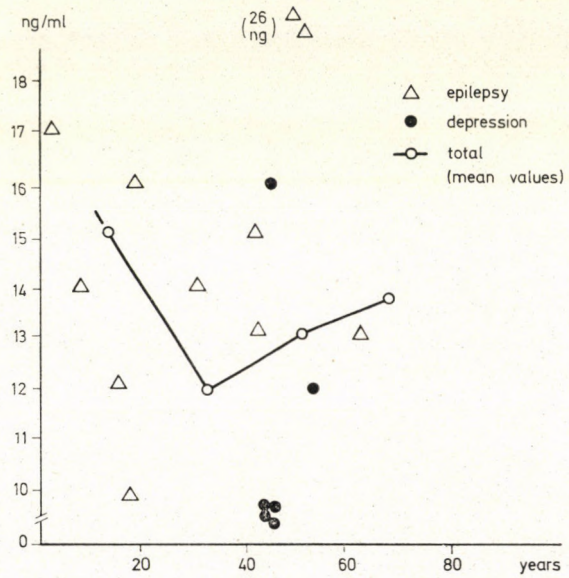

Fig. 2. 5-HIAA-level in CSF in depressive and epileptic cases

Table II

5-HIAA level in CSF in neuropsychiatric conditions

	Condition	Depression	Other psychiatric conditions	Epilepsy	Organic cerebral lesion	Other neuro-psychiatric condition		Drug-free
5-HIAA	Range of variations	4—16	5—30	8—26	4—30	5—32		4—28
	Mean ± S. D.	8.1 ± 4.5	13.1 ± 6.3	15.4 ± 5.0	15.3 ± 7.0	13.8 ± 6.2	±6.9	12.6
	Number of patients	6	13	11	29	35	21	
	Mean age	(48)	(49.8)	(31.8)	(47.4)	(50)	(38.7)	

Lumbar fluids (83), cisternal fluids (12).

* 2 patients with grave brain-stem lesion (5 and 6 ng/ml), 1 patient with spinal cord compression (8 ng/ml), 1 patient with taboparesis (7 ng/ml).

The difference between the means for the depressive and the epileptic cases is significant statistically ($p < 0.05$). The differences between the means for the other groups are not significant statistically.

Values very close to these levels were found in the organic cerebral lesion group (15.3 $\pm$ 7.0). The values for the groups "other psychoses" and "other neuropsychiatric syndromes" were lower, and still more so in the drug-free groups (12.6 $\pm$ 6.9). However, the differences between the groups were not

Table III

Loading tests

Patients	Loading	5-HIAA level in CSF, μg/ml
1. (F. J.)	probenecid (2.5 g/16 hr)	130
2. (P. Gy.)	tryptophan (6 g/16 hr)	31
3. (H. A.)	tryptophan (6 g/16 hr)	16

significant. Comparative analysis of the results showed that, with reference to the graph representing the age-distribution of the whole series, the levels measured in the depressive psychosis group were significantly lower than in the epilepsy group where the 5-HIAA was high in the majority of cases (Fig. 2).

In the "organic cerebral lesion" group the lowest levels were found in two patients with grave brain-stem lesion, in one with compression of the spinal cord and in one with taboparesis. Tryptophan loading performed in two cases yielded 31 μg/ml in the first case (neurosis) and 16 μg/ml in the other (epilepsy). (See Table III).

Probenecid loading performed in one case (Parkinson's syndrome) gave an excessively high value (130 ng/ml).

Discussion

Analysis of the present data has been focussed on the groups of depressive illness and of epilepsy. Though we had few depressive cases, there is fairly consistent evidence of decreased CSF-concentrations of 5-HIAA in depressive illness. In the present cases, the mean level was low but plotting against the graph of age distribution of the whole series brought out the differences very clearly. ASHCROFT et al. (1966) found a mean of 10.3 ng/ml in neurological conditions. The low 5-HIAA level in depressive illness may be due to a decrease of serotonin turnover in the limbic system, but a possible impairment of the transport mechanisms in depressive illness must also be taken into account (GLEN et al., 1968), therefore the low level may be connected with some disorder of 5-HIAA transport.

There is no published evidence of any characteristic change in the CSF 5-HIAA level in epilepsy. Actually, our aim of studying the 5-HIAA level in

epileptics has been to establish the mean in a fair number of epileptic cases within an unselected neuropsychiatric population, rather than to find changes specific of epilepsy. In fact, in the epilepsy group the level was found to be closest to that in the organic cerebral lesion group. This makes it justified to ascribe the elevation of the 5-HIAA levels to similar factors in both groups. The barrier-function may be impaired in both cases. It cannot be excluded that the increase in the 5-HIAA concentrations is secondary to the seizures. On the other hand there is experimental evidence that serotonin promotes the tendency to convulsions (TRUITT and EBERSBERGER, 1962), though other observations suggest that serotonin does not affect the seizure-threshold (SCHAEPDRYVER et al., 1962). The observations of PFEIFER et al. (1966) suggest that of the two compounds noradrenalin and serotonin, it is noradrenalin which is more cosely affecting te predisposition of the brain to convulsive activity. It may be assumed that a high susceptibility to convulsious requires reduced levels of both amines. Involvement of the brain-stem in the production of generalized epileptic seizures has been confirmed by electrophysiological evidence. On the other hand, the characteristic changes in the cerebral concentration of 5-hydroxyindole point to an essential biological role of this compound. It has been shown (COSTA et al., 1958, etc.) that the 5-hydroxyindole concentrations of the brain-stem are fairly high. This has been confirmed by our findings in human autopsy material.

Informative evidence relative to the high concentrations of 5-HIAA of CSF in epilepsy has been provided by the studies of ANDERSON et al. (1962), diphenylhydantoin having been found to increase the cerebral serotonin level parallel with the anticonvulsant action.

BOWERS and GERBODE (1968) noted a relationship between age and the concentration of monoamine metabolites in human CSF, expressed by an U-shaped curve. Earlier authors (JOHANSSEN, and ROOS, 1967) failed to demonstrate any relationship of this kind. The connection between age and the CSF metabolite levels is emphasized by the low 5-HIAA and HVA-concentrations in the CSF, thus the very reverse of those predicted on the basis of the U-shaped curve, in certain conditions affecting the elderly, in particular in the senile and presenile types of dementia (GOTTFRIES et al., 1969, 1970). We also sought to gain information on the value of loading tests. Though a definite increase in the 5-HIAA level in response to tryptophan-loading was demonstrable in our studies too, the results have yet to be confirmed by repeated self-controls. By extending our studies to tryptophan loading we sought to gain information, in accordance with the findings of MOIR et al. (1970), on the integrity of the pathways of cerebral 5-hydroxyindole metabolism. Probenecid loading was reported to provide information of similar value in respect of the transport mechanisms. The value of this test in Parkinson's disease has been discussed by SONNINEN (1971) on the grounds of a well-documented analysis.

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SERUM GLYCOPROTEIDS IN CONNECTIVE-TISSUE DISEASES AND OTHER PATHOLOGIC CONDITIONS

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The levels of individual serum glycoproteids and of their carbohydrate components have been studied in connective tissue diseases and other pathologic processes. The changes of the glycoproteids displayed no consistent pattern in any of these processes. There was a rising tendency of the carbohydrate components. It is pointed out that glycoproteid estimation must be selective to be of clinical aid.

The glycoproteids belong to the essential elements of the connective tissue ground substance. Therefore, every process involving the ground substance is bound to affect the metabolism of glycoproteids and to modify their serum level.

Quantitative changes in the carbohydrate groups of serum glycoproteids, in particular in protein-bound hexose, hexosamine and sialic acid, have extensively been studied in various pathological conditions including diseases of connective tissue, of the kidney, and malignant tumours. All these processes are associated with an increase in concentration of the carbohydrate component [2, 7, 22]. Despite the widely diverse physical, chemical, biological and immunological properties of the human serum glycoproteid fractions, they all include the carbohydrate groups referred to above [8, 14]. Consequently, quantitative changes in the concentration of these carbohydrate components do not allow conclusions as to changes in the individual glycoproteids. Therefore, the different serum glycoproteids have to be studied individually. This would also offer a promising angle of approach to the aetiology and pathogenesis of certain pathological processes. Investigations in this field are still in the early stage [2, 10, 11, 15, 19].

In the present study, sera of patients suffering from different diseases have been studied for individual glycoproteids, estimating the protein-bound hexose, hexosamine, sialic acid and seromucoid levels.

Material and method

The sera for study have been obtained from patients of both sexes admitted in the period 1970 to 1972. Their age ranged from 24 to 76 years.

From the sera of 14 patients (12 females, 2 males) with systemic lupus erythematosus the concentrations of IgG, IgA, IgM, coeruloplasmin, α_2-macroglobulin, transferrin and β_1C-

globulin have been determined on 16 occasions by the radial immune diffusion technique of MANCINI et al. [9], using monospecific sera supplied by the Institute of Human Vaccine Production and Research, Budapest.

The group of rheumatoid arthritis included 12 patients (8 females and 4 males). There were 9 cases of chronic pyelonephritis, (5 females and 4 males) their sera having been studied on 11 occasions. Malignant disease was represendited by 17 cases (13 females and 4 males). In the majority of cases, the protein-bound carbohydrate components were also determined. Hexose was estimated according to STARY et al. [16]; hexosamine according to SWANN and BALÁZS [17];

Table I

Serum glycoproteid concentrations (mg/100 ml)

		Controls	SLE	Rheumatoid arthritis	Chronic pyelonephritis	Malignant disease
IgG	n	17	16	12	11	17
	x′	1200	1719	1526	1194	1488
	t		2.19	1.61	0.42	1.93
	p		<0.05	>0.05	>0.05	>0.05
IgA	n	17	16	12	11	16
	x′	210	344.8	324.8	362.7	296
	t		2.25	2.09	2.13	2.3
	p		<0.05	<0.05	<0.05	<0.05
IgM	n	17	16	12	11	16
	x′	130	213.2	229.5	163.5	190.8
	t		2.5	2.24	1.74	2.3
	p		<0.05	<0.05	>0.05	<0.05
Coeruloplasmin	n	17	16	12	11	16
	x′	31	51.3	53.3	43.6	54.6
	t		2.39	2.24	1.78	2.7
	p		<0.05	<0.05	>0.05	<0.01
α_2 macroglobulin	n	17	16	12	11	16
	x′	244	266.1	295.1	395.6	339.0
	t		0.96	1.48	2.06	2.31
	p		>0.05	>0.05	<0.05	<0.05
Transferrin	n	17	15	12	9	15
	x′	295	228.9	294.4	236	244.9
	t		2.09	0.04	1.5	1.77
	p		<0.05	>0.05	>0.05	>0.05
β_1C globulin	n	17	16	12	11	16
	x′	110	90.8	123.8	119.2	134.8
	t		1.83	1.15	0.92	1.77
	p		>0.05	>0.05	>0.05	>0.05

sialic acid according to WARREN [21]; seromucoid according to WEIMER and REDLICH-MOSHIN [23]. Pooled normal human sera from 100 blood donors served as controls for radial immune diffusion and sera from 16 healthy subjects (12 females, 4 males) for the biochemical procedures. The relative values yielded by radial immunodiffusion were translated into absolute figures on the basis of reference sera (HYLAND, KALLESTAD). In the case of transferrin and of β_1C-globulin, reference sera were not available, therefore the results obtained were referred to the generally accepted control values by given BECKER et al. [1]. For statistical analysis the method of SCHILDER [13] and the t test were applied.

Results

With the exception of the SLE-group, no significant elevation of IgG was found. The concentration of IgA was significantly increased in all four groups. Elevation of IgM was not significant in renal disease, while it was significant in the other groups. The changes in coeruloplasmin were similar. The concentration of α_2-macroblobulin was significantly increased in renal disease and in tumours. The behaviour of transferrin requires special comment. A significant fall was noted in SLE, but there were two cases each in the renal and the malignant group where the values were too small for quantitative evaluation. If these values had been accessible to quantitative analysis, a significant fall could have been registered in these groups, too. The con-

Table II

Serum-protein-bound carbohydrate concentrations (mg/100 ml)

		Controls	SLE	Rheumatoid arthritis	Chronic nephritis	Malignant disease
Hexose	n	16	15	10	9	13
	x′	104.1	145.8	163.5	156.8	143
	t		4.1	4.28	5.12	2.98
	p		<0.01	<0.01	<0.001	<0.05
Hexosamine	n	16	15	10	9	13
	x′	71.8	88.2	111.1	101.1	89.5
	t		2.4	4.5	3.36	2.04
	p		<0.05	<0.01	<0.01	>0.05
Sialic acid	n	16	15	10	9	13
	x′	61.1	83.6	107.2	93	92.5
	t		3.98	6.25	4.76	4.58
	p		<0.01	<0.001	<0.01	<0.001
Seromucoid	n	16	15	10	9	13
	x′	12.6	19	31.1	18.8	36.3
	t		1.25	5.3	1.8	4.6
	p		>0.05	<0.001	>0.05	<0.001

Table III

Direction of changes in serum glycoproteid concentration

	SLE	Rheumatoid arthritis	Chronic pyelonephritis	Malignant disease
IgG	+	⊕	⊕	⊕
IgA	+	+	+	+
IgM	+	+	⊕	+
Coeruloplasmin	+	+	⊕	+
α_2-m	⊕	⊕	+	+
Transferrin	−	⊖	⊖	⊖
β_1C-globulin	⊖	⊕	⊕	⊕

Table IV

Direction of changes in serum glycoprotein carbohydrate components

	SLE	Rheumatoid arthritis	Chronic pyelonephritis	Malignant disease
He	+	+	+	+
Ha	+	+	+	⊕
Si	+	+	+	+
Se	⊕	+	⊕	+

+ significant increase; − significant decrease.
⊕ non-significant increase; ⊖ non-significant decrease.

centration of β_1C-globulin remained largely unaffected in all groups. Among the protein-bound carbohydrate components, hexose and sialic acid revealed significantly increased levels in all groups. Elevation of the hexosamine level was not significant in malignant disease, but significant in the other groups. Elevation of seromucoid concentrations was significant in rheumatoid arthritis and in malignant disease (Tables I to IV).

Discussion

CLEVE and STROHMEYER [2] estimated the concentration of α_1-acid GP, α_2-macroblobulin and Gc-globulin in a large patient material comprising different types of disease. An elevation of α_1-acid GP concentration was found in cholangitis, tumours and systemic disease, in contrast to hepatic cirrhosis where the values tended to decline. The α_2-macroglobulin concentration was increased in cholangitis, malignant disease, SLE, liver cirrhosis and hepatitis.

A reduction in the Gc-globulin concentration was found in hepatic disease. SNYDER and ASHWELL [15] determined 15 different glycoprotein fractions in a few patients with malignant disease and in a control group including healthy subjects and patients with various diseases. Though the small number of cases and the heterogeneity of the groups call for caution in evaluating the results, certain facts none the less deserve notice. In seven of the glycoproteids under study (transferrin, α_2-macroglobulin, Gc-globulin, IgA, IgD, IgG, IgM) normal concentrations were noted all throughout, in contrast to three others (α_1-acid GP, coeruloplasmin, α_1-antitrypsin) which revealed increased concentrations in all patient groups. The concentrations of two (haptoglobin, haemopexin) were increased in malignant disease, those of three others ($\alpha_{2\,HS}$-GP, β_2-GPI, prealbumin) were decreased in malignant disease, and unchanged in other conditions. It is remarkable that the seromucoid group which has been regarded as homogeneous is actually made up of different glycoproteids displaying unaltered, increasing or diminishing concentrations.

MIESCH et al. [10] studied a large patient material, but here too the individual categories were heterogeneous, and all the controls were 20 year old healthy males. It deserves notice that a normal α_1-antitrypsin level was never found under pathological conditions, and α_2-macroglobulin level remained practically unaffected in all cases. An increase of some extent was demonstrable in malignant disease and in hepatitis. There are data showing an increase in some glycoproteid fractions in rheumatoid arthritis [12].

ZAWADSKI and EDWARDS [24] observed hypertransferrinaemia in patients with iron-deficiency anaemia. Increased serum haptoglobin levels were found in patients with myocardial infarction, cerebrovascular accidents and peripheral arterial disease [18]. HEVÉR et al. [6] as well as TÓTH et al. [20] measured the haptoglobin level on the basis of the haemoglobin-binding capacity and the coeruloplasmin level by colorimetry. The haemoglobin-binding capacity of serum was found to increase parallel with the progression of the malignant process, the findings in leukaemia were similar. No change was found in the distribution of the haptoglobulin types. With the exception of multiple myeloma, all malignant processes of the blood-forming system were associated with an increased coeruloplasmin level. The increase was most characteristic in Hodgkin's disease, where the level was related to the severity of the process. An increase was found also in patients with malignant tumour. FEHÉR et al. [4, 5] examined the level of individual glycoproteids and of protein-bound carbohydrate components in various types of chronic hepatitis and in cirrhosis of the liver, and revealed different alterations in the different conditions.

In view of the small number of cases the present results are far from conclusive, none the less they allow to stress some points. The most remarkable feature emerging from the findings is the differentiated character of the

changes in the levels of the various glycoproteids. Among the immunoglobulins, the glycoproteid character of which is common knowledge, IgG revealed a significantly increased concentration only in SLE whereas the IgA level was increased in all four disease groups. The concentration of transferrin was significantly reduced in SLE, while it was practically unaffected in rheumatoid arthritis and tending to decrease in the two other groups. The concentration of β_1-C-globulin remained practically unaffected; its changes, if any, were not of the same direction. This was especially clear in comparison with the changes in the protein-bound carbohydrate components, as these were marked by a rising tendency in all patient groups, attaining the level of significance in the majority of cases. It is hardly possible to establish any distinct relationship between the changes in the concentration of glycoproteids and in those of the protein-bound carbohydrate components. This may have had various causes. The glycoproteids under study represent but a minority of those occurring in the serum. Moreover, the individual glycoproteids differ widely in carbohydrate content. The cases making up the individual patient groups were inadequate in number and homogeneity. In addition, refinement of the procedures applied for the study of these compounds would be desirable in order to increase their reliability and informative value.

The present results, without being conclusive, clearly indicated that, in order to be of any diagnostic help or to add to the understanding of different pathological processes, studies must be directed at individual glycoproteids. It also emerged from the results that in connective tissue diseases the constellation of serum glycoproteids is different from that in renal or in malignant disease. Differences have also been found in SLE and in rheumatoid arthritis. These facts clearly point to the biological significance of glycoproteids and their possible involvement in the pathogenesis of various pathological conditions.

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IMMUNOGLOBULINS ON THE SURFACE OF LYMPHOCYTES IN AUTOIMMUNE DISEASES

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The immunoglobulin-bearing lymphocytes of patients with autoimmune disease have been studied by direct immunofluorescence. In the cases where the autoimmune process revealed signs of activity, an increase in the proportion of B-lymphocytes was found, whereas in inactive cases the distribution of B-lymphocytes was similar to that in the controls. The increase in the proportion of B-cells is attributed to a reduction in the absolute number of circulating T-lymphocytes.

The human lymphocytes represent a double population, one of which displays a high density of immunoglobulins on its surface [2, 6, 14]. Earlier studies [1, 5, 12] have furnished indirect proof of the existence of membrane bound immunoglobulins. Recently, the presence of immunoglobulin receptors on the surface of lymphocytes has been confirmed in animal experiments [4, 7—11, 13], similar results were obtained with human lymphocytes [2, 3, 6]. Immunoglobulin-bearing cells of this kind are now considered to be identical with B-lymphocytes (bursa-dependent or bonemarrow-derived cells).

Pamichail et al. [6] found an increase in the proportion of these immunoglobulin-bearing cells in the active periods of rheumatoid arthritis. In view of the primary pathogenetic role of cell-mediated immune reactions, thus of the T-cellpopulation in autoimmune diseases, it was deemed of interest to examine the proportion of the lymphocytes in the active and inactive phase of autoimmune disease.

Material and method

Seventysix patients with autoimmune disease and 10 controls were studied. Autoimmune diseases were as follows: 58 (40 active) cases of SLE, 10 cases (6 active) of rheumatoid arthritis, 1 active case of thyroiditis, 2 (1 active) cases of lupoid hepatitis, 1 inactive case of polymyositis. In 4 cases the autoimmune process indistinct (2 active). Activity was based on clinical signs and on the evidence of immunological and other laboratory tests (BSR, ANF and DRF titre, LE-cell phenomenon, etc.). The absolute leukocyte count was determined in every case.

Direct immunofluorescence was carried out as follows: the buffy coat of 10 ml heparinized blood was aspirated, centrifuged at 200 g, washed with TC-199 medium, and resuspended in 4 ml of TC-199 medium. The suspension was layered onto 2 ml of a mixture (1077spec. gravity) of 6.3% Ficoll^{R} (Pharmacia) and 10% Uromiro^{R} (Bracco), then centrifuged at 4°C at 1000 g for 15 min. The cell-layer at the interface was aspirated, washed twice with TC-199 medium, centrifuged at 200 g, finally made up to a dilution of 10^{7} cells per ml. Then, 0.05 ml of the lymphocyte suspension was incubated with an equal amount of fluoresceine isothiocyanate

— conjugated AHG (Hyland goat antihuman IgG, IgA, IgM) under repeated shaking at room temperature for 30 minutes. Concentration of the conjugate was 1 mg/ml. After incubation the cells were centrifuged at 200 g, washed twice in TC-199, and resuspended in 0.1 ml glycerol buffer (equal parts of isotonic saline with 0.01 *M* phosphate buffer and glycerol). The cells were examined in suspension using a Zeiss (Jena) microscope (light source HBO 200, excitant filter BG 12, barrier filter OG 1).

Results

The immunoglobulin-bearing B-cells revealed the common fluorescence pattern (Figs 1, 2). Their distribution is shown in Table I. In the inactive cases the proportion of B-cells was similar as in the controls. The results obtained in the controls were consistent with published data [6]. On the other hand, in the patients with signs of activity, an increase was found in the proportion of IgG- IgA- and IgM-bearing B-cells. The absolute lymphocyte counts are given in Table II. While in the inactive cases the absolute lymphocyte count was practically the same as in the controls, in the cases with signs of activity it was significantly reduced.

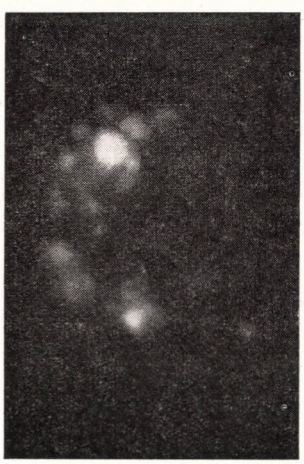

Fig. 1

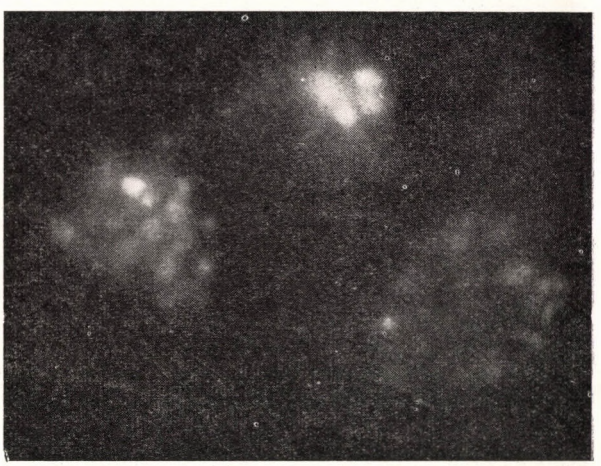

Fig. 2

Table I

	Immunoglobulin-bearing lymphocytes, per cent		
	IgG	IgA	IgM
Controls	15.4 (7—35)	5.9 (3—11)	5.5 (2—11)
Active autoimmune disease	25.4 (11—76)	12.8 (4—20)	8.6 (2—26)
Inactive autoimmune disease	16.2 (8—32)	6.2 (1—12)	5.9 (2—11)

In brackets: range.

Table II

Absolute lymphocyte count in active and inactive autoimmune disease

Controls	2100 (1800—2500) ml
Active cases	1300 (800—1600) ml
Inactive cases	2100 (1600—2500) ml

Discussion

A consistent proportion of the peripheral lymphocytes belongs to the B type. On the evidence of the present study, in inactive periods of autoimmune diseases, in the lymphocyte population the proportion of B-cells is similar as in healthy controls, and it increases with the activation of the immune process, parallel with the appearance of leukopenia. A significant fall in the absolute lymphocyte count was demonstrable in the present cases, too. This makes it obvious that the preponderance of B cells is a relative one. The fall in the number of T lymphocytes may be attributed to a fixation of the lymphocytes in the tissues in consequence of an activation of the cellular immune reactions. The leukopenia too might be due to an involvement of the lymphoid system by the autoimmune process.

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IMMUNOGLOBULINS IN SPUTUM AND SERUM IN IMMUNE DEFICIENT HAEMATOLOGICAL SYNDROMES (PARAPROTEINAEMIA, LYMPHATIC LEUKAEMIA)

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Immunoglobulins in serum and sputum have been estimated in 22 cases of immune deficiency secondary to haematological syndromes (lymphatic leukaemia, multiple myeloma) and in 25 patients with bronchitis. A significant decrease in serum IgA and IgM was found in the cases of immune deficiency. On the other hand, the sputum immunoglobulin levels were increased in comparison with those observed in the bronchitis group. IgG was moderately, secretory IgA and IgM were significantly, increased. The increase in the sputum immunoglobulin concentration in the cases of immune deficiency is regarded as a compensatory phenomenon.

The locally produced immunoglobulins occurring in biological fluids have been attracting increasing interest in recent years. The secretions of the respiratory and alimentary tracts have been found to contain all three main immunoglobulins in variable proportions [9, 13, 14, 15]. The predominant type of immuno-globulin demonstrable in biological fluids is the secretory IgA, a complex formed by the IgA molecule of two non-covalent bonds and by the transfer factor (secretory component, secretory piece) of 50,000 molecular weight [9]. The IgA molecule and the secretory component are formed independently of each other, the former in the plasma cells of the submucosa, the latter in the epithelial cells [21, 29, 30, 32]. Lately, in addition to the heavy, the light and the secretory polypeptide chains, a fourth chain, the J-chain (joining-chain) has been identified, first in the secretory IgA, subsequently also in the IgM, molecule. Thus far, the J-chain has been found exclusively in polymeric IgA and IgM molecules. The J-component is of 20,000 molecular weight, it contains glutamic acid and aspartic in large amounts. Its in tramolecular site of junction is not yet known. Anti-J-component antibodies have been demonstrated despite its week antigenicity. The J-components linked to IgA and to IgM differ in respect of their antigenic determinants [31a].

On the evidence of immunofluorescent studies, the ratio between the IgA producing plasma cells and the IgG producing immunocytes of the intestinal mucosa equals 20 : 1 and that between the IgA-producing and the IgM producing cells, 7 : 1 [32]. The bronchial mucosa forms prevalently IgA, to a lesser extent IgM [3, 27].

Secretory IgA is a bioactive protein of a sedimentation coefficient of 11S. In contrast to the plasma IgA polymers it is not affected by reducing agents. Locally formed IgA resists the digestive action of pepsin or trypsin as long as 24 to 72 hours without any loss of antibody activity. The polymeric structure of secretory IgA provides for a higher efficiency of its antibody activity [21, 25, 27, 28].

BRANDTZAEG et al. [6] attribute IgA and IgM of the biological fluids to active, IgG to passive transport. IgA and the secretory component are deposited in the form of a fine film on the surface of the intestinal mucosa, forming a barrier against the entry of pathogens [32, 1]. In fact, certain authors attribute to secretory IgA a larger part in this protective mechanism than to serum [23].

The functions attributed to the secretory immune system are as follows.

1. control of the normal bacterial flora of the respiratory, digestive and urogenital organs;

2. defence mechanisms against pathogens (secretory IgA being involved in various antiviral and antibacterial antibody functions [26]; BONOMO et al. [3] could demonstrate isoantibodies in the sputum by passive haemagglutination in a case of bronchitis after administration of typhoid vaccine;

3. the local immune system seems to be involved in the pathomechanism of autoimmune disease [2, 22, 32, 33].

Pathological conditions may affect the immunoglobulin composition of biological fluids and the plasmatic/secretory immunoglobulin ratio. Parallel measurements of immunoglobulins in serum and sputum have been carried out in congenital hypo- and agammaglobulinaemia, chronic bronchitis and bronchial asthma. Studies of this kind in antibody deficiency secondary to haemoblastoses have not been recorded in the literature. In earlier studies we have measured the serum and salivary immunoglobulins chiefly in IgA-deficient paraproteinaemia and in lymphatic leukaemia [23]. Despite a significant fall in serum IgA the salivary IgA level showed a moderate increase. The fact that in secondary antibody deficiency sinobronchial infections are frequent, has made us to study the immunoglobulins in the sputum with reference to those in serum and the digestive tract.

Material and method

Parallel serum and sputum immunoglobulin measurements were carried out in 22 cases of lymphatic leukaemia and multiple myeloma; 25 cases of chronic obstructive respiratory disease served as controls. The radial immunodiffusion procedure of MANCINI, CARBONARA and HEREMANS [18] was used with monospecific anti-IgG (L-25B), anti-IgA (L-40B) and anti-IgM (L-147L) immune sera supplied by the Institute for Human Vaccine Production and Research, Budapest. From the serum to be examined fourfold dilutions were prepared whereas the sputum, after mechanical homogenization, was placed undiluted into the antigen recipient. In view of the relatively low immunoglobulin concentrations of the sputum in comparison with that of the serum, the immune sera were diluted tenfold for IgA and IgG, and fivefold for IgM, so as to render the procedure more sensitive.

Results

Control group. The serum and sputum immunoglobulin levels were measured in 25 adults with obstructive respiratory disease. A decrease in serum-IgG was found in contrast to an elevation of serum IgA and IgM levels.

Table I

Mean serum and sputum immunoglobulin values in the controls and in the immune deficient haematological group

	Serum		
	IgG mg per 100 ml	IgA mg per 100 ml	IgM mg per 100 ml
Control group (chronic bronchitis, bronchial asthma), n = 25	1302 ± 61.7	258.9 ± 29.8	236 ± 29.4
Immunodeficient haematological group (multiple myeloma, lymphatic leukaemia), n = 22	1160 ± 131.6	119.1 ± 18.4 $p < 0.001$	79.9 ± 12.6 $p < 0.001$
Normal subjects, n = 60	1570 ± 52	210 ± 27	193 ± 15

	Sputum		
	IgG mg per 100 ml	IgA mg per 100 ml	IgM mg per 100 ml
Control group (chronic bronchitis, bronchial asthma), n = 25	30.08 ± 6.15	25.9 ± 2.53	4.71 ± 1.1
Immunodeficient haematological group (multiple myeloma, lymphatic leukaemia), n = 22	48.21 ± 12.3	45.2 ± 7.1 $p < 0.01$	11.1 ± 2.52 $p < 0.05$

Versus the normal controls, these changes were not significant statistically. (The normal serum levels had been established earlier in 60 healthy adults.)

In the immune deficient haematological group, the serum IgG level was moderately, the IgA and IgM levels were significantly reduced as compared with the control values. On the other hand, the sputum IgG level showed a moderate, while IgA and IgM a significant, increase (Table I).

No correlation was found between the immunoglobulin concentrations of serum and sputum in the individual patients. Reduced serum immunoglobulin levels were associated with increased, normal or reduced sputum immunoglobulin levels.

Fig. 1 presents the serum and sputum immunoglobulin values found in the same patients. While in the control group the serum immunoglobulin levels generally attained, and in the case of IgA and IgM even slightly exceeded, the normal mean values, in the group of haematological diseases the serum immunoglobulin levels were far below the normal mean values. On the other hand, in

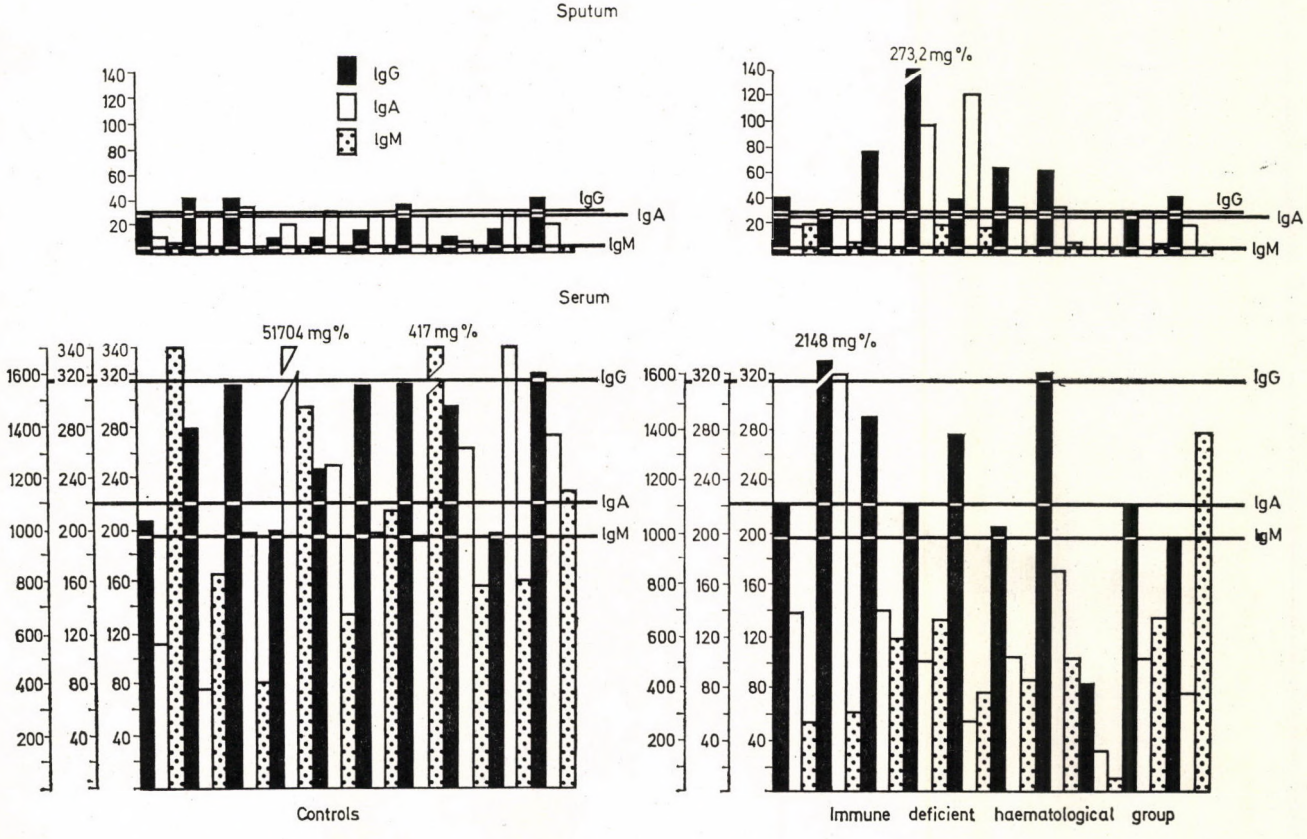

Fig. 1. Serum and sputum immunoglobulins in individual patients. Horizontal lines represent for the sputum the mean values for the control group of bronchitis; for serum, those of the normal controls

the immunodeficient haematological cases the sputum immunoglobulin levels were higher than in the controls. The serum and sputum immunoglobulin levels showed no correlation whatever.

Discussion

The lack of a correlation between the serum and secretory immunoglobulin concentrations has been shown by several authors [5, 10]. Serum immunoglobulin deficiency does not exclude the production of secretory immunoglobulins, and conversely, impairment of the local immunoglobulin producing system is compatible with normal serum immunoglobulin concentrations [4, 13, 28].

TÜRK and WIERBITZKY [33] found a complete or incomplete deficiency of IgA and IgG in the bronchial secretion of children with recurrent bronchitis and pneumonia, despite normal serum immunoglobulin concentrations. According to BÜRGI et al. [8] and BÜRGI and MEDICI [9], acute bronchitis and recurrences of bronchial asthma are associated with increased sputum IgA levels. In congenital immunedeficient conditions, deficiency of locally formed IgA and of the "secretory component" may well be responsible, in addition to serum immunoglobulin deficiency, for the recurrent infections [11, 17]. BONOMO et al. [3] found no IgA in serum or sputum in individual cases of this type. In a case reported by BUCKLEY et al. [7], increased serum IgA concentrations were associated with secretory IgA deficiency. In the cases of IgA deficient ataxia teleangiectatica reported by SOUTH et al. [27] and MCFAIRLIN et al. [27] salivary IgA and the secretory factor levels were normal. MARTINEZ-TELLO et al. [19] identified the secretory component in bronchial epithelium and secretions in the neonatal period and in agammaglobulinaemia. EIDELMAN et al. [12] found in the intestinal mucosa a normal IgA producing immunocyte population in total serum IgA deficiency. In atrophy of the intestinal mucosa, immunoglobulin secretion may be deficient and IgA transport deranged, in consequence of the deficient piece determinant production by the affected intestinal epithelium. The serum IgA level is increased in inflammatory processes of the intestinal mucosa and in malabsorption syndromes of children [16, 24]. Immunofluorescent studies in adults have revealed certain correlations between the number of immunoglobulin forming cells of the intestinal tract and the increase in the serum IgA level [12].

We have been considering the possibility of an enhanced compensatory activity of the local immune system ever since our earlier observations in haemoblastoses associated with immune deficiency, where the salivary IgA level was normal or increased despite a significant reduction in serum IgA [23]. Parallel estimations of sputum and serum immunoglobulins then revealed in

the sputum a significant increase in IgA and IgM, and lesser increase in IgG. The present results thus seem to confirm our earlier supposition.

The studies of THOMPSON [30] and of BRANDTZAEG [6] have furnished direct proof of a compensatory production of secretory IgA. THOMPSON studying the IgA-levels and the piece determinants in saliva and in duodenal secretions of IgA-deficient children with coeliac disease accompanied by recurrent infections was able to identify piece determinants attached to IgM in the duodenal juice. BRANDTZAEG [6] in an IgA deficient patient with plasmocytoma involving the parotid, found higher IgG concentrations in the intact parotid stroma than in normal individuals, while the salivary IgA and IgM levels were twice as high as the IgG level. On these grounds, this author attributed IgA and IgM to active, IgG to passive, transport.

According to the current view, in IgA deficiency the deficient glandular IgA forming immunocytes are replaced by IgM immunocytes [4, 6]. The question whether in IgA-deficient conditions the immunocytes of the whole secretory system are replaced by IgM-immunocytes, still remains open.

The present study has furnished only indirect evidence in support of the compensatory function of the local immune system in antibody deficiency. It is expected to obtain direct proof of this compensatory mechanism from immunofluorescent studies.

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RECENSIO

Zs. Hollán: Haemoglobinok és haemoglobinopathiák. (Haemoglobins and haemoglobinopathies.) Publishing House of the Hungarian Academy of Sciences, Budapest 1972. 280 pages, numerous illustrations.

Integration of experimental research with clinical observations ensures a fruitful approach to many problems of medicine. This is a truth which could not be illustrated more convincingly than by the results of haemoglobin research the current status of which is presented in this volume. Disclosure of the spatial structure of the haemoglobin molecule and of its diverse variants and its synthesis, together with a closer insight into its physico-chemical as well as immunological and genetic implications bear testimony to the productivity of interdisciplinary (chemical, genetic, immunological, clinical, etc.) research.

This book represents a successful blend of theory and practice. It gives a lucid, comprehensive survey of the structure and function of the haemoglobin molecule (Chapter I) and of its polymorphism (Chapter II). It describes the various haemoglobinopathies (Chapters III to VI) and finally discusses the prevalence and their genetic aspects of haemoglobinopathies in certain populations. The abundance of practical examples illustrating the issues under discussion contributes to the understanding of the pathology of the individual haemoglobinopathies and offers helpful clues to the selection of appropriate therapy.

The student of a book of this kind will be usually most attracted by the chapter which suits best his own line of study or his personal sphere of interest. Here the reviewer has some difficulty in deciding to which part of the book he would give preference, because all chapters offer equally attractive reading. If he none the less points out those on thalassaemia and on the haemoglobinopathies associated with polyglobulism, this is because, in his view, these are the most illustrative of the results of joint experimental and clinical studies.

The large number of graphs and illustrations, as well as an exhaustive list of 550 references add to the merits of the book.

Gy. Rappay

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РЕЗЮМЕ

ОПРЕДЕЛЕНИЕ ЛИЗОСОМАЛЬНЫХ ФЕРМЕНТОВ ПРИ НЕЙРОЛОГИЧЕСКИХ И ПСИХИАТРИЧЕСКИХ ЗАБОЛЕВАНИЯХ

Ш. ТОТ, И. УЙВАРОШИ и Б. УНГАР

Авторы проводили определения Кислой фосфатазы в вентрикулярном, цистернальном и поясничном ликворе больных, страдающих нейрологическим или психиатрическим заболеванием. От случая к случаю они определяли также активность нейтральной протеазы. При оценке данных более чем 100 больных, относящихся к определенной группе заболеваний, можно установить, что в ликворе можно выявить кислую фосфатазу, однако, ее активность составляет только одну десятую часть величин, измеряемых в сыворотке. В противоположность этому в ликворе здоровых лиц нельзя выявить активности нейтральной протеазы.

Сильно повышенная активность кислой фосфатазы наблюдается при опухолевых заболеваниях центральной нервной системы, как в случае доброкачественных, так и злокачественных опухолей. Наивысшие величины были получены в ликворе, взятом за несколько часов до наступления клинической смерти, в то время как после удаления опухоли активность кислой фосфатазы показывает тенденцию к нормализации.

В ликворе больных опухолями при физиологической величине pH появляется активная протеаза. Этот факт выдвигает предположение о том, что опухоль, якобы, оказывает в центральной нервной системе отдаленное действие нового типа — ферментативное отдаленное действие.

ИССЛЕДОВАНИЕ КИНЕТИКИ ИОДНЫХ ГОРМОНОВ ПРИ ПОМОЩИ СПЕКТРОФОТОМЕТРИЧЕСКОГО МЕТОДА

К. Г. БАРТА

Исследования авторы были направлены на выяснения соотношения распределения активных иодных гормонов и их связи кинетики иодных гормонов организма. Автор асследовал далее вопрос сколько из двух, трех или четырех иодных субституетнов в молекуле иодного гормона являются мечен иодом. В своем исследовании автор определял соотношение активных и неактивных компонентов иодного гормона щитовидной железы методами ауторадиографии и абсорбционной спектрофотометрии. На основании полученных результатов можно делать следующие установления:

1. Процентное отношение иодных гормонов за время исследования было постоянным и совпадало с таковым, приведенным в литературных данных.
2. Соотношение активных гормональных компонентов колебается вокруг величины, характерной для динамического равновесия. Из этого можно делать выовды относительно кинетики образования и мобилизации иодных гормонов.
3. Полученные результаты подтверждают то предположение, что в молекуле иодного гормона размещается только один активный иодный субститует.
4. Каждый из активных компонентов иодного гормона достигает через 24 часа ту величину, которая характерная для динамического равновесия неактивных иодных гормонов.

КЛИНИЧЕСКИЕ И ГИСТОПАТОЛОГИЧЕСКИЕ ИССЛЕДОВАНИЯ ПРИ ТЕРАПЕВТИЧЕСКИХ ПОЧЕЧНЫХ ЗАБОЛЕВАНИЯХ IV. ВОПРОС СВЯЗИ МЕЖДУ ФИБРОЭЛАСТОЗОМ ПОЧЕЧНЫХ АРТЕРИЙ, ГЛОМЕРУЛОНЕФРИТОМ И ГИПЕРТОНИЕЙ

И. ВАРГА, Э. БЕРЕГИ

На основании изучения гистопатологических и клинических данных 55 почечных больных авторы пришли к выводу, что при терапевтических почечных болезнях фиброэластоз почечных артерий может возникать также и без наличия гипертонии, предположительно, в результате основной болезни. На возникновение сосудистого изменения влияет продолжительность существования болезни. Болезнь может встречаться в любом возрасте, у пожилых лиц она, однако, вероятно встречается чаще. Заболевание почечных артерий не параллельное явление со спазмом, со склерозом сосудов глазного дна. Нет характерной клинической картины, на основании которой можно было бы сделать выводы относительно состояния почечных сосудов.

Наряду с отрицательным результатом исследования под оптическим микроскопом электронномикроскопическим исследованием можно выявлять изменение базальной перепонки клубочков, эндотелия и эпителиальных клеток. Эти изменения монут быть последствием имевшего место гломерулонефрита или гипертонии и они объясняют протеинурию, наблюдающуюся при клубочках, кажущихся под опитческим микроском интактными.

ВЫЗВАННАЯ НОВЫМ МЕТОДОМ ЭКСПЕРИМЕНТАЛЬНАЯ ПОЧЕЧНАЯ ГИПЕРТОНИЯ У КРЫС

А. ФЕКЕТЕ, Е. ТАРЯН

Авторы разработали новый метод для вызывания экспериментальной почечной гипертонии у крыс: почечная артерия на одной стороне полностью перевязывается, на другой стороне не проводится никакого вмешательства. У подопытных животных, моложе одного года, за 30-недельный срок наблюдения авторы обнаружили прочное повышение кровяного давления приблизительно на 55 мм рт. ст., значительную гипертрофию сердца и почек.

ОБЪЕМ ПЛАЗМЫ, КРОВИ И ОБЩИЙ ОБЪЕМ ВОДЫ ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ ПОЧЕЧНОЙ ГИПЕРТОНИИ У КРЫС

А. ФЕКЕТЕ, П. МЕЗЕШ, Л. ТОТ

Односторонной перевязкой почечной артерии у крыс, при отсуствии вмешательства на контралатеральной стороне, авторы вызывали хроническую гипертонию. На 13-ой и на 30—37-ой неделях гипертонического состояния авторы обнаружили при неизмененном общем объеме воды понижение объема плазмы и крови. Уменьшение интравазального объема авторы считают результатом изменения до сих пор еще невыясненного механизма ренальной регуляции объема.

ДЕЙСТВИЕ ЦИТОСТАТИЧЕСКИХ ПРЕПАРАТОВ НА ПРИРАСТАНИЕ ПАССИРОВАННЫХ ЧЕЛОВЕЧЕСКИХ АМНИОНАЛЬНЫХ КЛЕТОК

П. ГЕРГЕЙ, Г. САБО, Д. СЕГЕДИ, Б. ФЕКЕТЕ, Д. ПЕТРАНИ

Автори описывают быстрый и простой проводимы in vitro цитостатический тест. Суть метода заключается в определении при помощи ???? клеток, меченых Cr^{51}. понижения способности прирастания человеческих амниональных клеток под влиянием цитостатического препарата. Авторы расматривают возможность связи между ингибированием прирастания и возникновением метастазов.

ИССЛЕДОВАНИЕ ИММУНОГЛОБУЛИНОВ, ГЛИКОПРОТЕИДОВ И АНТИГЕНА АВСТРАЛИЯ ПРИ ХРОНИЧЕСКИХ ПЕЧЕНОЧНЫХ БОЛЕЗНЯХ

Я. ФЕХЕР, Л. ЯКАБ, И. СИЛВАШИ

Авторы определяли радиальным иммунодиффузионным методом в сыворотке крови хронических концентрацию иммуноглобуринов IgG, IgA и IgM, а также церулоплазмина, альфа-2-макроглобулина, бета-I-C глобулина и трансферрина. Далее они исследовали пассивным гемагглютинационным методом титр гуморальных антител, реагирующих с белковыми антигенами клеточных ядер, митохондриев и растворимых белков, полученных из аллогеновой печени, а также они определяли микрометодом связывания комплемента антиген Австралия в сыворотке больных и титр антител против него. Авторы обнаружили, что при хроническом персистирующем гепатите IgA и IgM, а при хроническом агрессивном гепатите и при циррозе печени/содержание всех трех иммуноглобулинов достоверно выше, чем в контрольных случаях. При хроническом агрессивном гепатите наиболее выраженное повышение Ig*G* Концентрация церулоплазмина в сыворотке и альфа-2 макроглобулина повышены параллельно со степенью воспаления в строме печени; концентрация бета-I-C глобулина и трансферрина не повышены в оцениваемой мере. Антиген Австралия и гуморальные антитела авторы шаходили чаще всего при хроническом агрессивном гепатите.

ИНДУЦИРУЮЩИЕ ГИПЕРСЕКРЕЦИЮ ГЛОБУЛИНЫ В КРОВИ БОЛЬНЫХ ЯЗВОЙ ДВЕНАДЦАТИПЕРСТНОЙ КИШКИ

(На основании доклада, сделанного на конгрессе терапевтов в 1972 г.)

Ш. ДОБИ, Д. ПЕТРАНИ

Причина гиперсекреции, существующей в значительной части случаев язвы двенадцатиперстной кишки, нарушающей известные регуляторные механизмы, все еще не выяснена. Глобулиновая фракция в крови гиперацидных больных язвой желудка провоцировала в семи из семнадцати случаев значительную гиперсекрецию в желудке крыс. Этим возникает возможность, что в деле гиперсекреции соляной кислоты в желудке в определенной части роль может играть стимулирующий фактор иммуноглобулинового типа, возможно, аутоиммунный процесс.

ИССЛЕДОВАНИЕ МЕТАБОЛИТА СЕРОТОНИНА В ЛИКВОРЕ

Д. МОЛНАР, А. ФОДОР, И. КАРЦАГ, А. СИЛАДИ, И. УЙВАРИ

Авторы изучали на состоявшем из 95 больных смешанном найро-психиатрическом больничном материале, содержание 5-HIES в ликворе. На основании своих исследований авторы установили, что в различных возрастных группах концентрация 5-HIES различная и разброс также довольно значительный. Величины в молодом и в пожилом возрастах сравнительно более высокие, в среднем возрасте — ниже, но разница средник величин не является достоверной. Среди отдельных групп больных, наименьши оказалось содержание 5-HIES в ликыоре в группе больных депрессией (8,1 $\pm$4,5 нг/мл), сравнительно более высокое содержание авторы определяли в группе больных эпилепсией и «органическими церебральными поражениям» (15,4$\pm$5,0 и 15,3$\pm$7,0 нг/мл). Ниже концентрации содержания в этих двух группах больных оказалась концентрация 5-HIES в группе, не получавшей медикаментов. (Разница недостоверная), у двух больных с тяжелым поражением ствола мозга, у одного больного с компрессией спинного мозга и у одного больного табо-параличном авторы нашли крайне низкие величины содержания 5-HIES в ликворе (5—8 нг/мл).

Авторы приобрелы опыт исследованиями нагрузкой триптофаном и пробеницидом, надеясь на то, что они обогатят шкалу клинико-биохимических методов.

ИЗМЕНЕНИЯ КОНЦЕНТРАЦИИ ГЛИКОПРОТЕИДОВ СЫВОРОТКИ ПРИ СОЕДИНИТЕЛЬНОТКАННЫХ БОЛЕЗНЯХ И ПРИ ДРУГИХ ПАТОЛОГИЧЕСКИХ ПРОЦЕССАХ

Л. ЯКАБ, Я. ФЕХЕР

Авторы исследовали изменения концентрации гликопротеидов сыворотки и их углеводных компонентов при заболеваниях соединительной ткани и при других патологических процессах. Они констатируют, что исследованные гликопротеиды сыворотки при отдельных патологических процессах меняются не одинаковым образом. Повышающийся характер концентрации углеводных компонентов гликопротеидов сыворотки одинаковый. Авторы указывают на то, что индивидуальное определениегликопротеидов сыворотки может оказывать и используемую в клинике помощь.

ПОВЕРХНОСТНЫЕ ИММУНОГЛОБУЛИНЫ ЛИМФОЦИТОВ ПРИ АУТОИММУННЫХ БОЛЕЗНЯХ

П. ГЕРГЕЙ, Д. СЕГЕДИ, Г. САБО, Б. ФЕКЕТЕ, Д. ПЕТРАНИ

Авторы исследовали прямым иммунофлюоресцентным методом носящие иммуноглобулины лимфоциты больных, старадающих аутоиммунными болезнями. У аутоиммунных больных с активным заболеванием авторы наблюдали увеличение процента клеток В. Неактивные больные давали такие же результаты, как и контрольные лица. Увеличение процентного соотношения клеток В авторы привели в связь с уменьшением абсолютного числа циркулирующих клеток Т.

СОДЕРЖАНИЕ ИММУНОГЛОБУЛИНОВ В МОКРОТЕ И В СЫВОРОТКЕ ПРИ ГЕМЕТОЛОГИЧЕСКИХ БОЛЕЗНЯХ, СОПРОВОЖДАЮЩИХСЯ ДЕФИЦИТОМ ИММУНОГЛОБУЛИНОВ (ПАРАПРОТЕИНЕМИИ, ЛИМФОИДНАЯ ЛЕЙКЕМИЯ)

Ж. МИСЛАИ, А. ПАТАКФАЛЬВАИ, И. БАРАНЯИ, Д. КЁВЕШИ, Т. ЯВОР

Авторы определяли содержание иммуноглобулинов в мокроте и в сыворотке 22 больных, страдающих синдромом вторичного недостатка в антителах (хроническая лимфоидная лейкемия, миелома) и 25 больных бронхитом, У больных с недостатком в иммуноглобулинах величины концентрации IgA и IgM по сравнению с нормальными контролями достоверно понизились. Содержание иммуноглобулинов в мокроте было повышенным по сравнению с величинами бронхитической группы. Количество IgG было умеренно, количество IgA и IgM — достоверно повышено. Авторы считают повышение содержания иммуноглобулинов в мокроте больных с иммунным недостатком компенсаторным.

IMMUNOLOGICAL ASPECTS OF ALLERGY AND ALLERGIC DISEASES I—II

edited by *E. Rajka* and *S. Korossy*

Vol. I: Studies in Experimental Immunology
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The *first volume* deals with the general principles of experimental immunology and allergology. The various factors which have a part in the antigen-antibody reaction and which set the course of up-to-date diagnostics are discussed in several papers. The most recent results of research in the fields of transplantation and tumour immunity are described.
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PATHOPHYSIOLOGY AND PHARMACOLOGY OF CAPILLARY RESISTANCE

by *M. GÁBOR*

In the first part of the monograph the physiological and pathological aspects of capillary resistance are treated. The different methods used for measuring capillary resistance are described followed by the discussion of the mechanism of petechia formation. Daily and seasonal fluctuations of capillary resistance as well as its age, diet-and physical impact-(heat, u.v. and X-ray irradiation) dependent changes are given extensive treatment. The author also considers the methods of determining capillary resistance in the inner organs of experimental animals and in the conjunctiva. Separate chapters have been devoted to the changes in capillary resistance due to stress and to the practical implications, respectively. Various diseases in which capillary resistance might be changed, mostly decreased, are described. In the second part all those drugs which may influence capillary resistance, are surveyed. The last part of the monograph provides information on the hormonal and other control mechanisms regulating capillary resistance.

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Zusammenfassung

Die plasmaeiweißgebundene und die freie Harnsäure wurden mittels Sephadex-Gelfiltration getrennt. Bei der Kontrollgruppe betrug die Menge der eiweißgebundenen Harnsäure 20,3% der Gesamtharnsäure, während bei den Gichtkranken diese Zahl 16,9% ausmachte. Mittels ^{14}C-2 Harnsäure ließ sich im Normalplasma eine Eiweißbindung von ähnlicher Größenordnung nachweisen. Bei Arthritis urica beträgt die zum Plasmaeiweiß gebundene Harnsäureaktivität nur 46% der Norm. Das Plasma des unbehandelten Gichtkranken ist zur Bindung größerer Harnsäuremengen fähig, als das der mit Allopurinol behandelten Patienten.

Bei Hyperurikämie ist die absolute Menge der gebundenen inaktiven Harnsäure, trotz der niedrigeren Bindungsproportion signifikant höher, als bei der Kontrollgruppe. Die normalen Plasmaeiweiße sind ihrer harnsäurebindenden Fähigkeit zufolge bis zu einem gewissen Grade fähig die Gewebeausfällung der Harnsäure zu hemmen.

Unter den mittels Papierelektrophorese getrennten Plasmaeiweißfraktionen bindet sich die markierte Harnsäure zu den Globulinen α_2 und β_1.

Die Pathologie der Gicht, dieser altbekannten Krankheit, ist auch heute noch größtenteils ungeklärt. Nach dem zweiten Weltkrieg stieg die Häufigkeit der Krankheit in bedeutendem Maße an, sodaß selbst die atypischen Fälle erkennt werden können. Dieser Umstand lenkte unsere Aufmerksamkeit auf die Klärung einiger Fragen der Pathogenese.

Das grundlegende diagnostische Kriterium der Krankheit ist die Erhöhung des Plasma-Harnsäurespiegels. Die freie, nicht eiweißgebundene Harnsäure ist bekanntlich jener Faktor, durch den die Gewebeausfällung beeinflußt wird. Das Ziel unserer Untersuchungen war die Klärung jener Frage, ob sich in bezug auf die harnsäurebindende Kapazität der Plasmaeiweiße von Normalpersonen und Gichtkranken irgendwelche Unterschiede nachweisen lassen. Laut der Literaturangaben ist die Harnsäure-Proteinverbindung von unterschiedlicher Größenordnung. SHEIKH und MOLLER [10] fanden mittels Ultrafiltration insgesamt 5% der Plasmaharnsäure in gebundener Form, während ALVSAKER [1] die Proportion der Verbindung im Normalplasma auf etwa 25—30% schätzte.

Im Laufe unserer Untersuchungen trachteten wir die folgenden Fragen zu klären:

1. Die Größe des plasmaeiweißgebundenen Anteils des Plasma-Harnsäuregehalts.

2. Besteht ein Unterschied in bezug auf die Bindung des ^{14}C-2-Harnsäuremoleküls zum Plasmaeiweiß zwischen Normalpersonen, behandelten und unbehandelten Gichtkranken?

3. Genaue Verteilung der eiweißgebundenen ^{14}C-2-Harnsäure und der freien Harnsäure.

4. Durch welche Plasmaeiweißfraktion wird die Harnsäure gebunden?

Untersuchungsmethoden

Der Plasma-Harnsäuregehalt wurde mit dem klassischen Verfahren von FOLIN und DENIS sowie mit der enzymatischen Methode (Uricase lyophilisiert praktisch wasserlöslich, Serva, Feinbiochemie) bestimmt [2, 4, 8, 9, 11]. Aus dem Extinktionswert des verdünnten Plasmas bei der Spektrophotometrie (Zeiss Spektrophotometer UV VSU2-P, Wellenlänge 290 mμ) wurde der aktuelle Harnsäuregehalt ausgerechnet. Zu Beginn wurden sämtliche Plasmaproben sowohl chemisch als auch enzymatisch analysiert. Da es sich jedoch anhand des Vergleichs herausstellte, daß die Ergebnisse der beiden Verfahren nahezu von identischer Größenordnung sind und daß die Harnsäuremenge, welche bei der chemischen Methode im Laufe der Eiweißausfällung verloren geht, nicht mehr als 0,1 mg% beträgt, haben wir des weiteren den Plasma-Harnsäuregehalt mit dem chemischen Verfahren und die niedrigeren Harnsäuremengen der fraktionierten Untersuchungen mit der enzymatischen Methode bestimmt.

Die Trennung der eiweißgebundenen und der freien Harnsäure erfolgte durch Chromatographie auf einer 240 mm hohen Sephadex-G-75-Säule nach PORATH und FLODIN [7], bei einer Trennungs-Molekulärgewichtsgrenze von 40 000 [3].

0,5 ml frisch gewonnenes und zentrifugiertes menschliches Plasma wurde in einer Azetatpufferlösung (0,05 M, pH 7,4) filtriert. Mittels eines automatischen Fraktionsempfängers wurden insgesamt 14 Filtrate zu je 2 ml gesammelt und der Eiweißgehalt spektrophotometrisch (Zeiss VSU2-P, Wellenlänge 260 mμ) bestimmt. Die Plasmaeiweiße waren in den Filtraten 3—8 vorzufinden. Die eiweißhaltigen Fraktionen wurden mit 50 γ Harnsäure (pro anal.) versetzt und der Gesamtharnsäuregehalt ebenfalls spektrophotometrisch bestimmt. Zunächst wurde das System mit 1 mg Urikase (Serva Feinbiochemie) versetzt und im Wasserbad bei 37°C 1 Stunde lang inkubiert. Die zurückgebliebene Harnsäuremenge wurde photometrisch gemessen. Die Konzentration der eiweißgebundenen Harnsäure wurde unter Berücksichtigung des Verdünnungsgrades aus dem Extinktionsunterschied ausgerechnet.

Die Vorversuche sprachen dafür, daß sich sowohl die angewandte Urikasemenge als auch die Inkubationszeit zum Abbau der jeweiligen Harnsäuremenge ausreichend waren.

Zur Untersuchung der Verteilung der gebundenen und freien Harnsäure diente ^{14}C-2 Harnsäure (Amersham, Searle, Des Plaines, Illinois). Das Harnsäure-Isotop (Reinheitsgrad: 98—99%) wurde in 0,05 M TRIS-Puffer ph 7,34 gelöst; die Lösung enthielt 2 μc je ml Harnsäure. 0,5 ml frisch gewonnenes, zentrifugiertes Plasma wurde mit Harnsäure von 0,2 μc Gesamtaktivität versetzt, sodann nach 30minütiger Inkubation bei +20°C, sodann in 0,05 M TRIS-Puffer (pH 7,34) auf einer Sephadex G-75-Säule filtriert. Aus 15 Filtraten zu 2 ml wurde nach vorangehender Eiweißbestimmung der eiweißhaltige Anteil gesammelt und die Filtrate wurden bei 80°C eingetrocknet. Zunächst wurde das Untersuchungsmaterial in 10 ml Packard-Insta-Gel Lösung gelegt und die Aktivität mit dem Packard-Tricarb Liquidszintillations-Spektrophotometer gemessen. Aus den Mittelwerten von 6 Parallelbestimmungen wurde die Aktivität der Blindprobe in Abzug gebracht und die endgültige Aktivität der einzelnen Proben auf 1 ml Plasma und 1 μc Harnsäureaktivität gerechnet in 10^3 c. p. m. ausgedrückt.

Die Bestimmung der Verteilung der eiweißgebundenen und freien Harnsäure unter Anwendung markierter Harnsäure unterschied sich von der oben beschriebenen Methode nur darin, daß die mittels Gelfiltration gewonnenen 15 Fraktionen einzeln eingetrocknet wurden und die Aktivität eines jeden Filtrats zur Bestimmung kam.

Die Lokalisation der Harnsäure-Plasmaeiweiß-Verbindung wurde papierelektrophoretisch bestimmt. 0,1 ml frisch entnommenes und zentrifugiertes Plasma wurde mit 0,2 μc markierter Harnsäure versetzt, und 1 Stunde lang bei Zimmertemperatur stehen gelassen.

Hiernach wurde 0,05 ml des markierten Plasmas in Veronal-Natrium-Puffer pH 6,8 bei 25°C auf WHATMANschen Papier Nr. 1 elektrophorisiert. Zwei Bestimmungen wurden parallel vorgenommen; das eine Präparat wurde mit Sauerfuchsin gefärbt, das andere blieb ungefärbt. Es folgte die prozentuale Eiweißbestimmung des gefärbten Papierstreifens, während das ungefärbte Präparat den einzelnen Fraktionen entsprechend in Streifen geschnitten wurde. Die markierte Harnsäure wurde mittels 0,1 n NaOH 12 Stunden lang ausgelöst, und nach Eluierung eingetrocknet. Des weiteren wurde die Untersuchung in der oben beschriebenen Weise durchgeführt. Die Ergebnisse werden in c. p. m. angegeben.

Ergebnisse

1. Zu Beginn haben wir die eiweißgebundene Harnsäure mit Sephadex-Gelfiltration bestimmt. So wurde das Plasma von 12 Gichtkranken und 10 gesunden, normourikämischen Personen analysiert. Vor der Gelfiltration fanden in sämtlichen Fällen Plasma-Harnsäure- sowie qualitative und quantitative Eiweißbestimmungen statt. Fälle mit pathologischen Proteinwerten wurden aus dem Untersuchungsmaterial ausgeschlossen. Da sämtliche unserer Gichtkranken Männer waren, wurden als Kontrollpersonen ebenfalls Männer gewählt.

Bei Normalpersonen betrug der eiweißgebundene Anteil des Gesamtharnsäuregehalts des Plasmas 20,3 $\pm$ 1,56%; bei den Gichtkranken 16,9 $\pm$ 0,6%. Der Unterschied erwies sich als signifikant ($p < 0{,}05$) (Abb. 1). Da der Plasmaharnsäurespiegel bei Normalpersonen 4,1 mg $\pm$ 0,02% und bei Gichtkranken 8,4 $\pm$ 0,85% betrug, konnte es sich nicht darum handeln, daß die Plasmaeiweiße der Gichtkranken nur geringere Harnsäuremengen zu

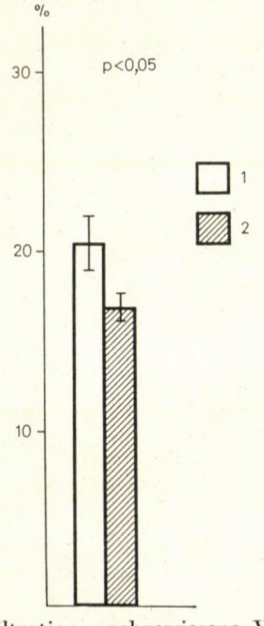

Abb. 1. Mittels Sephadex G-75-Filtration nachgewiesene Verteilung der eiweißgebundenen Harnsäure in Normal- und Gichtplasma
1 = Normalplasma, 2 = Gichtplasma

binden fähig wären. Die unterschiedliche harnsäurebindende Kapazität der Plasmaeiweiße von Normalpersonen und Gichtkranken ist somit, wenn man die prozentuellen Werte betrachtet, nicht absolut signifikant, dagegen ist in absoluten Werten die Eiweiß-Harnsäure-Verbindung stark signifikant. Die in der Gichtgruppe gefundene Konzentration der gebundenen Harnsäure (16,9%) entspricht einem 1,43 $\pm$ 0,05% Gehalt. Die 20,3% ausmachende Verbindungsproportion der Kontrollgruppe bedeutet einen Harnsäuregehalt von 0,83 mg $\pm$ 0,03% ($p < 0{,}001$).

Bei Gichtkranken wird somit eine geringere Proportion der Plasma-Gesamtharnsäure durch Eiweiß gebunden, die absoluten Werte zeigen aber, daß die Menge der freien und auch der gebundenen Harnsäure erhöht ist.

Die Ergebnisse dieser Untersuchungen informierten uns über die Menge der eiweißgebundenen Harnsäure mit nur annähernder Genauigkeit, zumal mit einer etwa 5%igen Fehlergrenze zu rechnen war.

2. Im Interesse der genaueren Analyse der Bindungsverhältnisse der Harnsäure bei gesunden, normourikämischen Personen sowie bei unbehandelten und behandelten Gichtkranken setzten wir unsere Untersuchungen mit ^{14}C-2-Harnsäure fort. 33 Plasmaproben wurden untersucht, 10 stammten von gesunden Personen und 23 von Gichtkranken. 12 der an Arthritis urica leidenden Patienten erhielten keinerlei Behandlung, 11 Patienten wurden täglich 200—400 mg Allopurinol (Zyloric, Borroughs-Wellcome) verabreicht. Die Ergebnisse veranschaulicht Abb. 2.

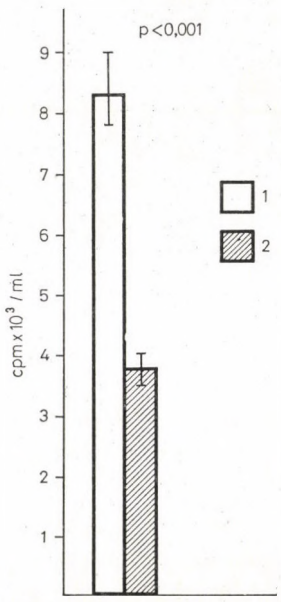

Abb. 2. Bestimmung der eiweißgebundene ^{14}G-2-Harnsäure in Normal- und Gichtplasm.a
1 = Normalplasma, 2 = Gichtplasma

Die Aktivität der eiweißgebundenen Harnsäure betrug in der Kontrollgruppe $8{,}34 \times 10^3 \pm 0{,}76$ c.p.m. und bei den behandelten und unbehandelten Gichtkranken $3{,}84 \times 10^3 \pm 0{,}66$ c.p.m. aus. Der p-Wert belief sich auf $<0{,}001$ und ist somit stark signifikant. Wird die markierte Harnsäureaktivität der Kontrollfälle als 100% betrachtet, so machte die eiweißgebundene Harnsäureaktivität der Gichtkranken nur 46% aus.

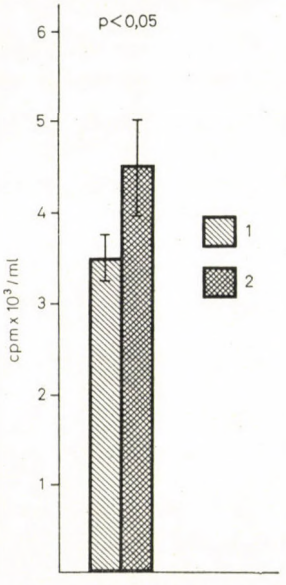

Abb. 3. Verteilung der eiweißgebundenen ^{14}C-2-Harnsäure im Plasma von unbehandelten und mit Allopurinol behandelten Gichtkranken
1 = behandelt, 2 = unbehandelt

Hiernach wurden die an Arthritis urica leidenden Patienten in zwei Gruppen eingeteilt: In 12 Fällen wurde im Zeitpunkt der Blutnahme bzw. während der 6tägigen vorangehenden Periode keine medikamentöse Behandlung angewandt, während 11 Patienten täglich 200—400 mg Allopurinol erhielten. Die Durchschnittswerte der Plasma-Harnsäurekonzentration machten in der unbehandelten Gruppe 9,6 mg% und in der behandelten 5,1 mg% aus. Die eiweißgebundene Harnsäureaktivität betrug in der unbehandelten Gruppe $4{,}55 \times 10^3 \pm 0{,}23$ c.p.m. und bei der behandelten $3{,}52 + 10^3 \pm 0{,}55$ c.p.m. Da $p < 0{,}05$ ausmachte, lagen die Werte an der Grenze der Signifikanz. (Abb. 3) Die im Prozentsatz der Normalkontrolle ausgedrückte Aktivität betrug bei den unbehandelten Patienten 54,5% und bei den behandelten 41,8%.

3. Die Diffusion der eiweißgebundenen ^{14}C-2-Harnsäure-Makromolekeln und der freien Harnsäure haben wir im Plasma von 3 Normalpersonen mittels Sephadex-Gelfiltration untersucht. Die Mittelwerte der einzelnen Fraktionen

sind in Abb. 4 dargestellt. Unter den 15 Filtraten zu je 2 ml enthielten die Filtrate 3—8 eine Aktivität; in den Filtraten 1—3 ließ sich keine nennenswerte Aktivität nachweisen. Die Aktivitätssteigerung meldete sich in Probe 4 und 5; von hier an stieg die Aktivität stufenweise an und erreichte ihre erste Spitze im Filtrat 8, d. h. im letzten, kein Eiweiß enthaltenden Röhrchen ($25{,}87 \times 10^3$ c.p.m.). Hier ließ sich somit 72% der eiweißgebundenen Aktivität nachweisen.

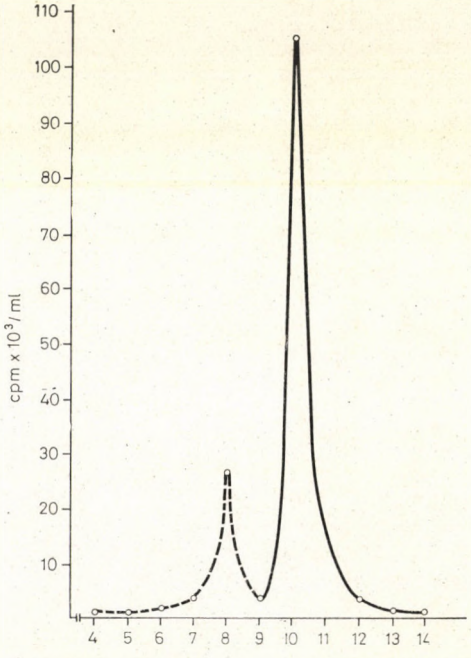

Abb. 4. Trennung der eiweißgebundenen und freien ^{14}C-2-Harnsäure im Normalplasma
- - - - - - - - gebundene Harnsäure, ———— freie Harnsäure

Nach der Fraktion 8 begann sich die Aktivität plötzlich zu vermindern, bis in den Filtraten 10 und 11 das Maximum der freien Harnsäure erschien. Auch auf diese Spitzenwerte folgte eine rasche Verringerung, das Filtrat 15 enthielt bereits keine Aktivität.

Während die eiweißgebundene Gesamtaktivität 35×10^3 c.p.m. ausmachte, betrug die Aktivität der freien Harnsäure 242×10^3 c.p.m. Die Ergebnisse unterscheiden sich wenig von den Werten der nicht fraktionierten Messungen. Mit der Isotoptechnik fanden wir im Normalplasma 20,9% gebundene und 79,1% freie Harnsäure.

4. Im Interesse der Feststellung der Lokalisation der Harnsäure-Eiweißbindung wurden 14 Plasmaproben analysiert (8 von unbehandelten Gichtkranken und 6 von Normalpersonen). In der Albuminfraktion der Gicht- und

Kontrollplasmen war nur eine dem Blindwert entsprechende Aktivität vorzufinden. Die c.p.m. in der α_1-Fraktion war in beiden Gruppen niedrig und obwohl sich die bei den Gichtkranken registrierten Werte als höher erwiesen, war der Unterschied nicht signifikant. Das Maximum der ^{14}C-2-Harnsäureaktivität konnte auf dem Gebiet der α_2- und β_1-Globuline ermittelt werden, wobei sich im Plasma der Gichtkranken signifikant höhere Werte nachweisen ließen ($p < 0{,}001$). Die sich in den β_2-Globulinen meldende Harnsäureaktivität war

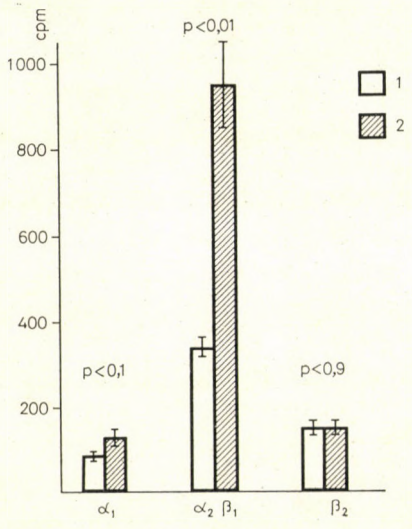

Abb. 5. Bindung von ^{14}C-2-Harnsäure durch die Plasmaeiweißfraktionen bei Gichtkranken und Normalpersonen
1 = Normal, 2 = Gicht

in beiden Gruppen identisch. Die Ergebnisse sind in Abb. 5 dargestellt. Die Gesamtmenge der freien Harnsäure trat auf den Randteilen der γ-Globulinfraktion in Erscheinung. Unsere Beobachtungen sprechen dafür, daß γ-Globulin die Harnsäure nicht bindet.

Besprechung

Die Zielsetzungen unserer Untersuchungen waren die Größenordnung bzw. Lokalisation der Harnsäure-Eiweißbindung zu klären, sowie auf die Fragen eine Antwort zu erhalten, ob die Änderung der Bindungsproportion von Harnsäure-Protein in der Entstehung des akuten Gichtanfalls sowie der Knochengelenks- und renalen Ausfällung irgendeine Rolle spielt.

Die Sephadex-Gelfiltration eignet sich zur Trennung niedermolekulärer Substanzen, wie z. B. harnsäurige Fraktionen. Mittels Gelfiltration lassen sich der freie und der gebundene Anteil der Harnsäure leicht trennen, während

durch Aktivitätsmessung die Genauigkeit der Bestimmung gesteigert wird. Durch Aktivitätsmessung kann die Zahl der sich zu den noch freien Stellen der Plasmaeiweiße bindenden ^{14}C-2-Harnsäuremolekeln bestimmt werden; diese Werte sind der absoluten Menge der eiweißgebundenen Harnsäure proportional.

Die obigen Untersuchungen ergaben, daß die Menge der eiweißgebundenen Harnsäure bei gesunden, nicht hyperurikämischen Personen 20,3—20,9% des Gesamtharnsäuregehalts ausmacht. Die normalen Plasmaeiweiße, was Menge und Zusammensetzung anbelangt, vermögen somit, ihrer harnsäurebindenden Fähigkeit zufolge in hyperurikämischen Zuständen die Menge der aktiven, freien, sich in den Geweben präzipitierenden Harnsäure gewissermaßen herabzusetzen, da sie die Umwandlung der freien Harnsäure in inaktive Harnsäure bewirken.

Bei hypoproteinämischen Zuständen, so z. B. bei sich zu Gichtniere gesellender Nephrose vermindert sich die Menge und wahrscheinlich auch die harnsäurebindende Kapazität der Plasmaeiweiße. Gleichzeitig steigt die Menge der aktiven freien Harnsäure sowohl in absolutem, als auch in relativem Maß an. Über die sich nebst dem pathologischen Eiweißbild entwickelte Harnsäure-Proteinverbindung wollen wir später berichten.

Durch Allopurinol, das heute als das meistverwendete Medikament bei der Behandlung von Arhtritis urica gilt, wird die Harnsäure aus der Eiweißbindung gelöst. Für das Gesagte liefert auch unsere experimentelle Beobachtung einen Beweis, laut der das Plasma der behandelten Gichtkranken zur Aufnahme kleinerer Harnsäuremengen fähig ist, als das der unbehandelten Patienten, d. h., daß die ^{14}C-2-Harnsäureaktivität des Plasmas der allopurinbehandelten Gichtkranken im Prozentsatz der normourikämischen Personen niedriger ist. Angesichts dessen, daß das Plasma der Gichtkranken weniger exogene Harnsäure zu binden fähig ist als das der Unbehandelten, steigt auf Wirkung von Allopurinol die Menge der freien, aktiven Harnsäure relativ an. Die Erscheinung liefert eine Erklärung unserer früherer klinischen Beobachtungen, laut deren sich in der Initialperiode der die Harnsäuresynthese hemmenden Behandlung, trotz der abnehmenden Tendenz des Plasma-Harnsäurespiegels, häufig akute Gichtanfälle melden [5, 6].

Im Laufe unserer Untersuchungen haben wir auch die Lokalisation der Harnsäure-Eiweißverbindung festgestellt. ALVSAKER [1] fand die Harnsäure größtenteils in Albuminbindung und in kleinerem Ausmaß in α_1-Globulinbindung. Unter den angeführten Versuchsbedingungen vermochten wir keine Harnsäure-Albuminverbindung zu beobachten, in den α_1- und β_2-Globulinen war die Verbindung von geringem Ausmaß, während sich die α_2-, β_1-Globulin-Harnsäure-Verbindung für ausgeprägt erwies. Die Abweichungen finden ihre Erklärung annehmbar in der Verschiedenheit der Methoden. Die α_2- und β_1-Globuline der Gichtkranken haben signifikant mehr Harnsäure gebunden, als die entsprechenden Globulinfraktionen der Kontrollpersonen.

Diese Messungen ermöglichten die Bestimmung der Lokalisation der Harnsäure-Eiweißbindung und es gelang uns mit der elektrophoretischen Methode den Umstand zu beweisen, daß die Plasmaeiweiße der Gichtkranken, insbesondere die α_2- und β_1-Globuline größere Harnsäuremengen binden, als die normalen Plasmaeiweiße.

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Herrn Prof. Dr. E. Endrőczi wollen wir für seine liebenswürdige Hilfe und seine wertvollen Ratschläge auch an dieser Stelle unseren herzlichen Dank zukommen lassen.

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CLINICAL AND HISTOPATHOLOGICAL STUDIES IN HUMAN RENAL DISEASE

V. HISTOLOGICAL, IMMUNOHISTOLOGICAL AND ELECTRON MICROSCOPIC FINDING IN FOLLOW-UP BIOPSIES

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In 70 patients with renal disease repeated biopsies were made. According to the findings, three groups could be distinguished, *viz.* 1) healing; 2) persistence of the changes revealed by the first biopsy; 3) deterioration. In Group 1 the only light microscopic indication of earlier glomerulonephritis was an occasional minor increase of the mesangial matrix and slight mesangial hypercellularity. The immunohistological findings were negative. Electron microscopic evidence of earlier glomerulonephritis was confined to an occasional fusion of the foot processes, the presence of lipid droplets in epithelial cells and vacuolisation of endothelial cell. In Group 2 the light microscopic findings were the same as at the first biopsy. In a number of cases immunohistology revealed the disappearance or decrease of the immune deposits; the basement membrane and the mesangium exhibited fibrin deposits. The relationships between fibrin and the course of glomerulonephritis are discussed. Electron microscopy revealed, in addition to immune deposits, numerous "moth-eaten" defects of the thickened basement membrane. In Group 3 there was light microscopic evidence of local adherence and hyalinization of glomerular tufts. The immunohistological findings were similar as in Group 2. The electron microscopic features included hyperplasia of the mesangial matrix, thickening of the basement membrane, fibrin deposits on the basement membrane and the mesangium, and "moth-eaten" defects of the basement membrane.

Needle biopsy of the kidney offers visual information on the renal disease at its onset and allows to follow its course and to correlate the clinical results of therapy with morphological evidence of the improvement, stagnation or deterioration of the process and thus to assess the further changes of the patient.

The present report sums up the results in 70 cases of renal disease of renal biopsy performed on 2, 3 or 4 occasions for a closer study of the successive stages of the process.

Material and Method

The material consisted of 70 patients with renal disease; in 64 of them two, in 5 three, and in 1 patient four successive renal biopsies were performed. Electron microscopic studies were carried out in 25 cases.

Renal needle biopsy was performed by means of an X-ray television screen, according to KARK and BUENGER [15], using a Franklin—Vim—Silverman needle.

The biopsy material was fixed immediately in 70% ethanol of 4 °C for light microscopic and immunofluorescence studies. For the latter, the specimens were processed by the method of SAINTE-MARIE [22], the sections were incubated with fluorescein-isothiocyanate-(FITC)-labelled anti-human IgG, anti-human complement (C'), anti-human IgM and anti-

human fibrin. (FITC-labelled antibodies were supplied by C. Hyland Travenol International, GMBH.) Specificity was checked by inhibition, the sections being incubated first with unconjugated, then with FITC-conjugated anti-human serum.

For light and fluorescence microscopic studies, an OPTON microscope with a HBO 200 W Osram lamp was used. Photographs were made with an automatic camera attached to the miscroscope. The blocks processed for the immunohistological studies served also for light-microscopy after staining with haematoxylin-eosin, PAS, Hart's, methenamine-silver (Jones), and Congo dyes.

For electron microscopy, the specimens after a short fixation in glutaraldehyde were fixed in osmium tetroxyde and embedded in araldite. The sections were prepared with a LKB-Ultratone III, contrasted with lead citrate and examined with a Hitachi-Hu 10, or a JEM 6 AS electron microscope.

Results

Table I shows the morphological and immunohistological findings of the first biopsy, compared with those of the subsequent biopsies. The terminology of CHURG, HABIB and WHITE [4] was adopted with the modification that the membraneous (extramembraneous or epimembraneous) nephropathies were subdivided on the basis of the light-microscopic and immunofluorescence findings into three types. In Type I, the light microscopic changes are confined to a fine thickening of the basement membrane with no spiky projections but immunohistology showed IgG and C' deposits on the glomerular basement membrane. Similar changes have been described by SEYMOUR et al. [23] in the early phase of epimembraneous nephropathy with slight or indistinct light-microscopic changes. BOHLE et al. [2] attributed these changes to acute membraneous glomerulonephritis. We could, however, furnish immunohistological evidence of massive IgG and C' deposits on the glomerular basement membrane even in this early stage (Fig. 1). In fact, we regard this finding as distinctive of membraneous glomerulonephritis Type I. Type II is

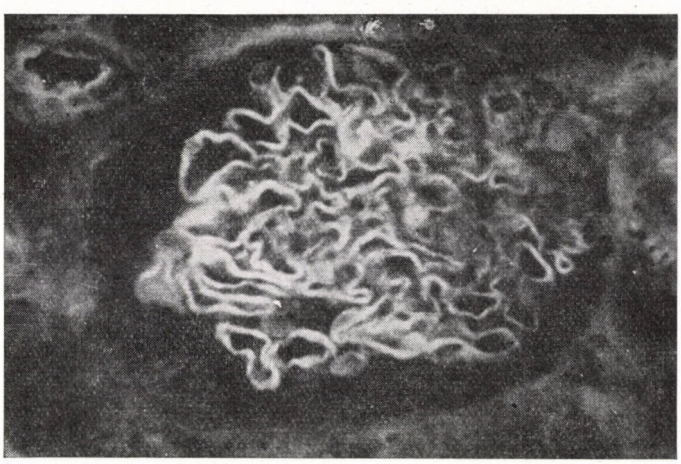

Fig. 1. Membraneous glomerulonephritis Type I. Light microscopic changes are confined to a slight thickening of the basement membrane. Immunofluorescence reveals massive IgG deposits on the glomerular basement membrane. (Incubated with FITC-conjugated anti-human IgG)

Table I

Histology first biopsy	Number of cases	Histology repeated biopsy		Number of cases	Immunohistology repeated biopsy Number of cases			
					in patches IgG, C'	fibrin only	no IgG, C'	unchanged IgG, C'
Membraneous glomerulonephritis I.	8		Membraneous glomerulonephritis II.	29	17	9	19	25
Membraneous glomerulonephritis II.	43	No change	Membraneous glomerulonephrits III.	10				
Membraneous glomerulonephritis III.	10		Membranoproliferative glomerulonephritis	2				
Diffuse proliferative glomerulonephritis including	9		With crescent Membraneous	1				
Membranoproliferative glomerulonephritis	6	Impairment	glomerulonephritis II. Membraneous glomerulonephritis III.	3 10				
Focal glomerulonephritis	2		Membranoproliferative glomerulonephritis	4				
With crescents	1		Chronic glomerulonephritis	1				
Associated vascular changes	6		Membraneous					
Associated pyelonephritis	3	Healing	glomerulonephritis I. Membraneous glomerulonephritis II.	6 3				
			Focalis glomerulonephritis	1				
		Pyelonephritis healed		3				
		Associated vascular changes		8				
		Associated pyelonephritis		2				

defined by the presence of spiky projections of the basement membrane in addition to immunological activity. Finally, the features marking Type III of membraneous glomerulonephritis include, in addition to the former changes a fusion and hyalinization of occasional glomerular loops and of individual glomeruli, in other words signs indicative of incipient chronicity but still too indistinct to warrant the diagnosis of chronic glomerulonephritis. Differentiation of these three types was expected to provide a basis for the therapeutic lines to be adopted. For instance, drug treatment of Type I is different from that of Type III. In accordance with the classification by CHURG et al. [4], the group of diffuse proliferative glomerulonephritis consideration was given to the mesangial, focal, crescent-forming and membranoproliferative types. There was no exudative type in our series. Nor did our repeated biopsy material include cases with minimal changes or focal sclerosing glomerular lesions. The cases with histological features of associated pyelonephritis have been set out separately in Table I because this type of pyelonephritis usually healed to adequate therapy. We found it important to indicate the number of cases in which vascular changes such as fibroelastosis or a lamellar hyperelastosis of the interlobular artery had been revealed by the first biopsy. In Table I, the findings of the last biopsy have been divided into three groups, *viz.* healing, no change (persistence of the changes revealed by the first biopsy) and impairment. The changes are also presented in which associated vascular changes or pyelonephritis had not been demonstrated before the second biopsy.

Healing of the process was revealed in 10 out of 70 cases. The histological types are shown in Table I. At the time of the first biopsy there were IgG and C' deposits on the basement membrane (Fig. 2) in membraneous glomerulo-

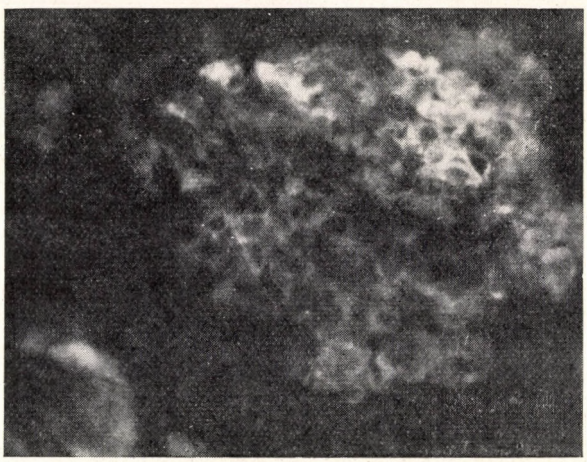

Fig. 2. Renal biopsy of 14 year old boy. First biopsy: light microscopical finding typical of membraneous glomerulonephritis, Type I. Immunofluorescence: massive IgG deposits of granular character on glomerular basement membrane. (Incubated with FITC-conjugated antihuman IgG)

nephritis I and II; electron microscopy revealed a thickening of the glomerular basement membrane with electrodense precipitates, in some cases on its subepithelial in some others on its subendothelial aspect (Fig. 3), moreover the foot processes were fused and the epithelial cells contained lipid droplets and vacuoles. In a case of focal glomerulonephritis, subendothelial focal thickening of the basement membrane and focal proliferative changes were noted.

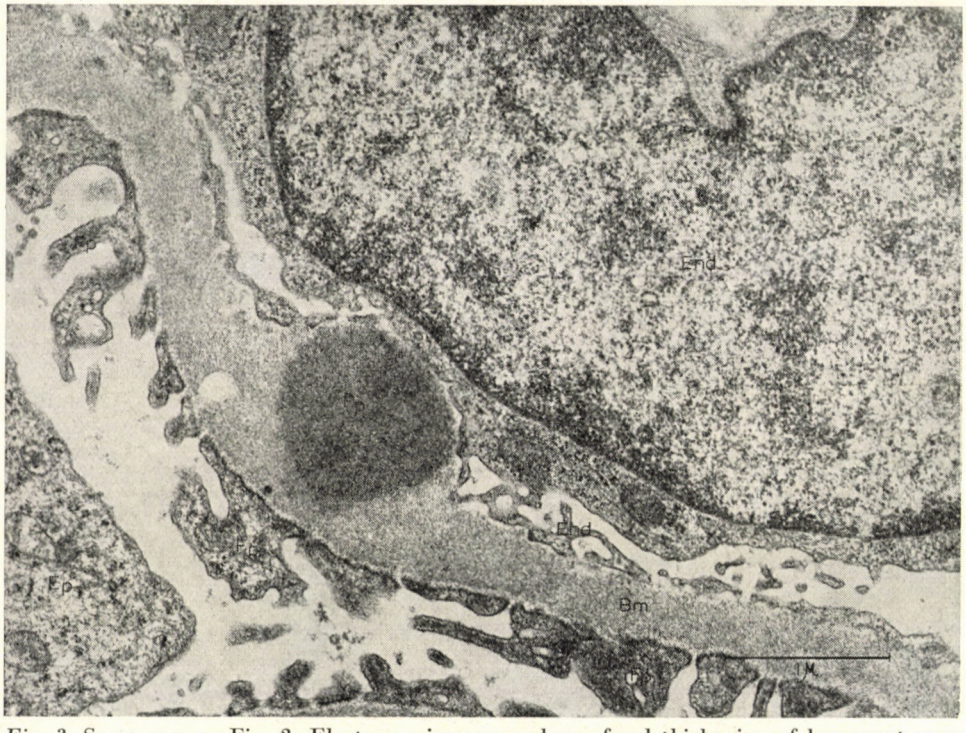

Fig. 3. Same case as Fig. 2. Electron microscopy shows focal thickening of basement membrane, subendothelial deposits of electrodense precipitate. ×36,750

In the cases, regarded as healed on the evidence of the last biopsy, the glomerular basement membrane had a membraneous appearence all throughout. The residual light microscopic changes consisted of a slight hyperplasia of the mesangial tissue and at the axial region there was a slight proliferation of mesangial cells. In a single case only did the residual vascular changes remind of the earlier inflammatory process. Immunohistology was negative in all cases. The residual electron microscopic changes were confined to a slight hyperplasia of the mesangial matrix (Fig. 4) and an occasional inhomogeneous thickening of the glomerular basement membrane which was free from immune deposits. There were still sporadic fusions of the foot processes. Some epithelial cells were marked by oedematous swelling or lipid droplets and the endothelial cells by vacuolation (Fig. 5).

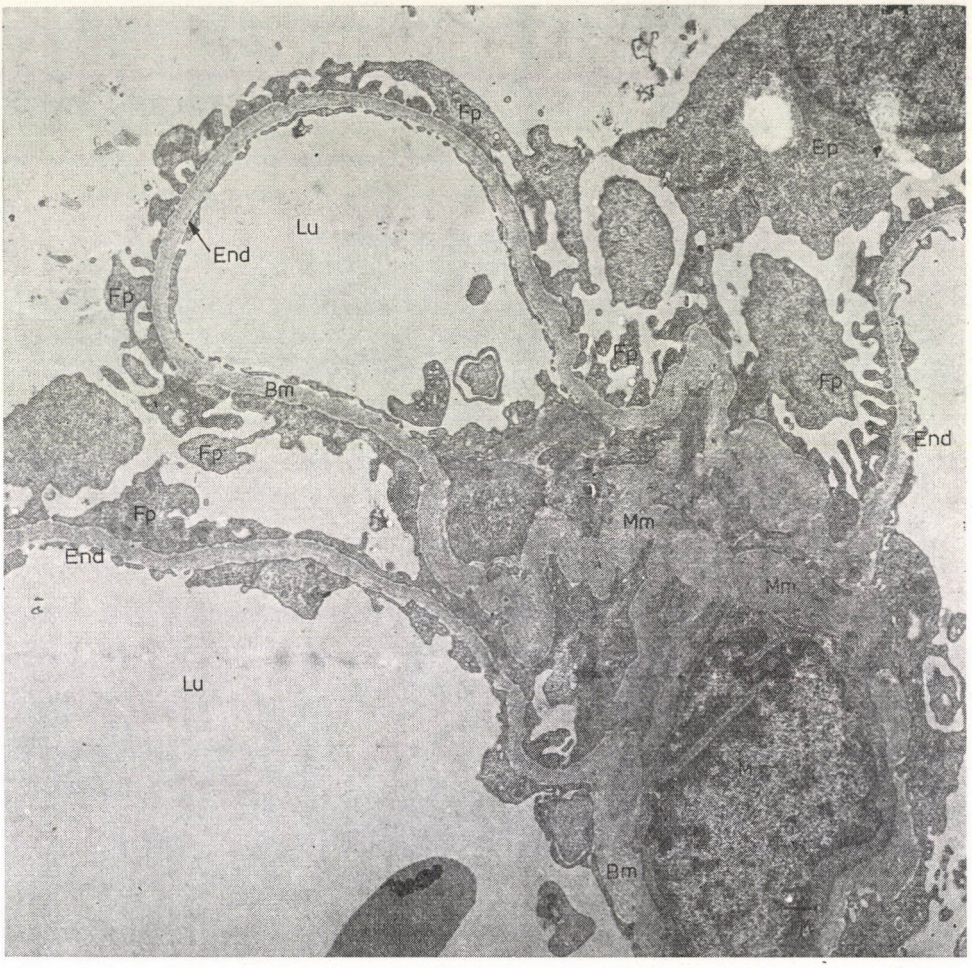

Fig. 4. Same case as Fig. 3. Repeated biopsy. Electron microscopy shows a thin basement membrane, occasional fusion of epithelial foot processes; focal hyperplasia of the mesangial matrix (×4,750)

In 42 cases, the light microscopic changes were similar to those revealed by the first biopsy. In 18 cases, the process tended to deteriorate, in other words, to assume chronic features. One case of focal glomerulonephritis had progressed into a diffuse glomerulonephritis. In 8 cases where no vascular changes had been present at the first biopsy, the repeated biopsy revealed fibroelastosis and lamellar hyperelastosis of the interlobular artery. In 2 cases, associated pyelonephritis had remained undetected until repeated biopsy. The immunohistological findings were similar in these two groups. In 9 cases, though there was a definite light microscopic evidence of glomerulonephritis, IgG and C' which had formed massive deposits at the time of the first biopsy

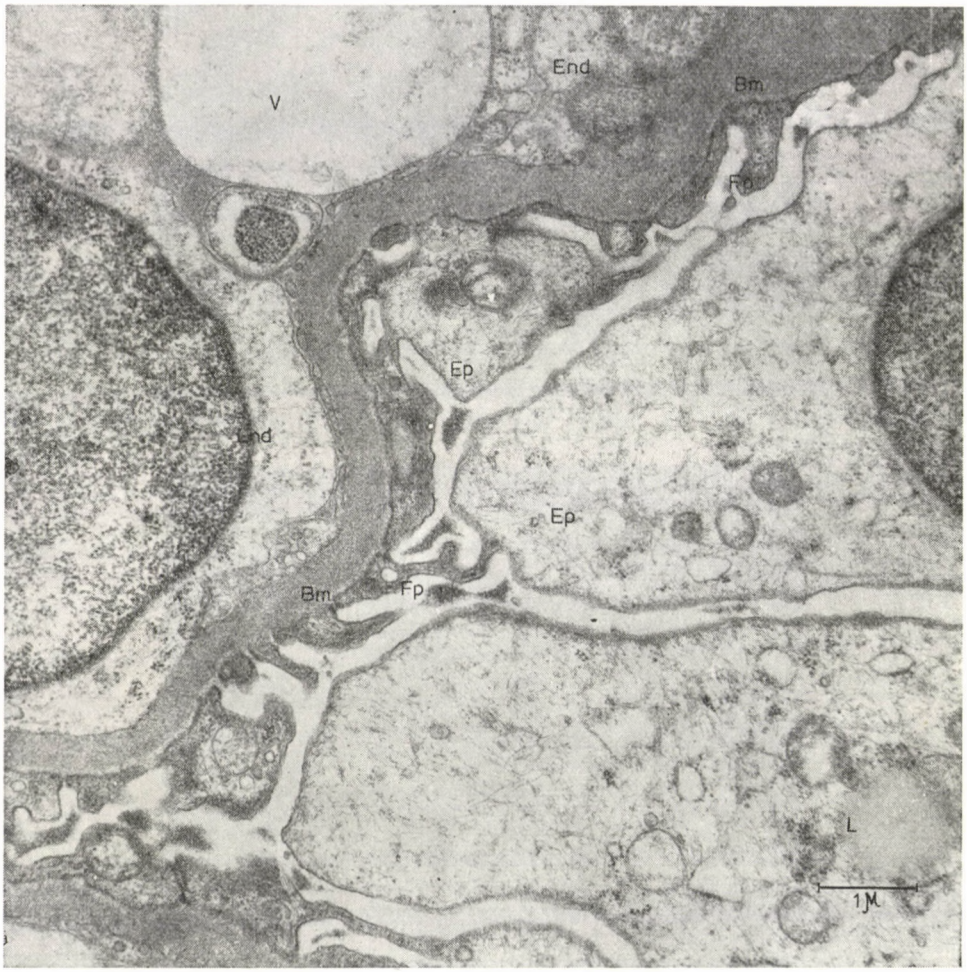

Fig. 5. Female patient of repeated biopsy. By light microscopy and of immunofluorescence the process had healed completely. Electron microscopy reveals a glomerular basement membrane of normal thickness; oedematous swelling of epithelial cytoplasm; occasional fusion of foot processes; swelling and vacuolation of endothelial cells (×17,000)

(Fig. 6), were no longer demonstrable. (In other cases, the negative results were indicative of a healed process.) While immunological activity was confined to small patches (Fig. 7) or was absent, in a number of cases granular fibrin deposits were demonstrable partly on the basement membrane, partly in the mesangium (Fig. 8). This raised the possibility that stagnation or deterioration of the process might be connected with the presence of fibrin. In 17 cases deposits of IgG and C' were confined to sporadic patchy areas, in 25 cases immunological activity had remained unchanged. As regards the factors accounting for the disappearance or decrease of IgG and C', two possibilities

Fig. 6. Male, 31 years. First biopsy. Glomerular basement membrane with massive IgG deposits. (Incubated with FITC-conjugated anti-human IgG)

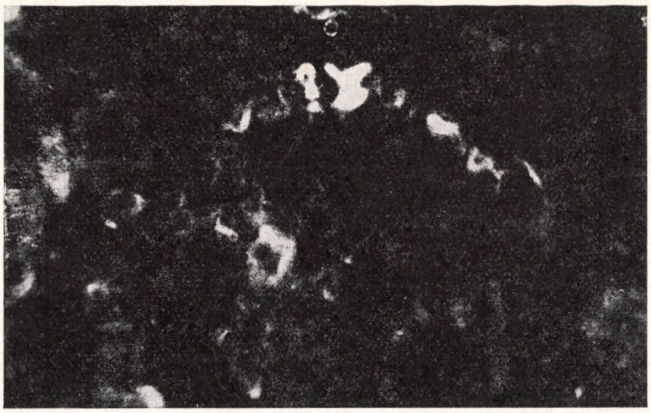

Fig. 7. Same case as in Fig. 6. Repeated biopsy. The glomerular basement membrane shows IgG only in patches instead of the former massive deposits. (Incubated with FITC-conjugated anti-human IgG)

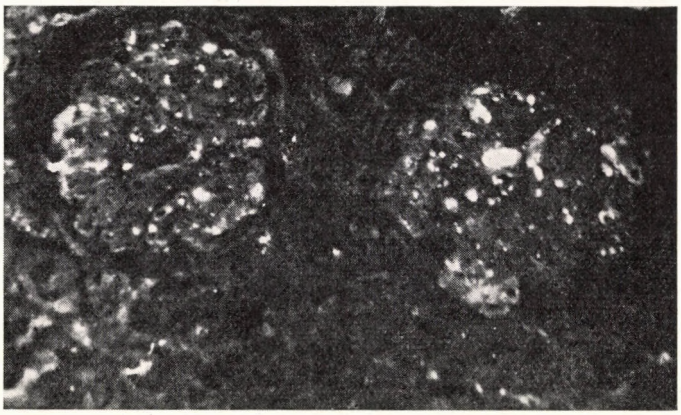

Fig. 8. Repeated biopsy, same case as in Fig. 6, shows massive fibrin deposits of granular structure on glomerular basement membrane and in mesangial connective tissue. (Incubated with FITC-conjugated anti-human fibrin)

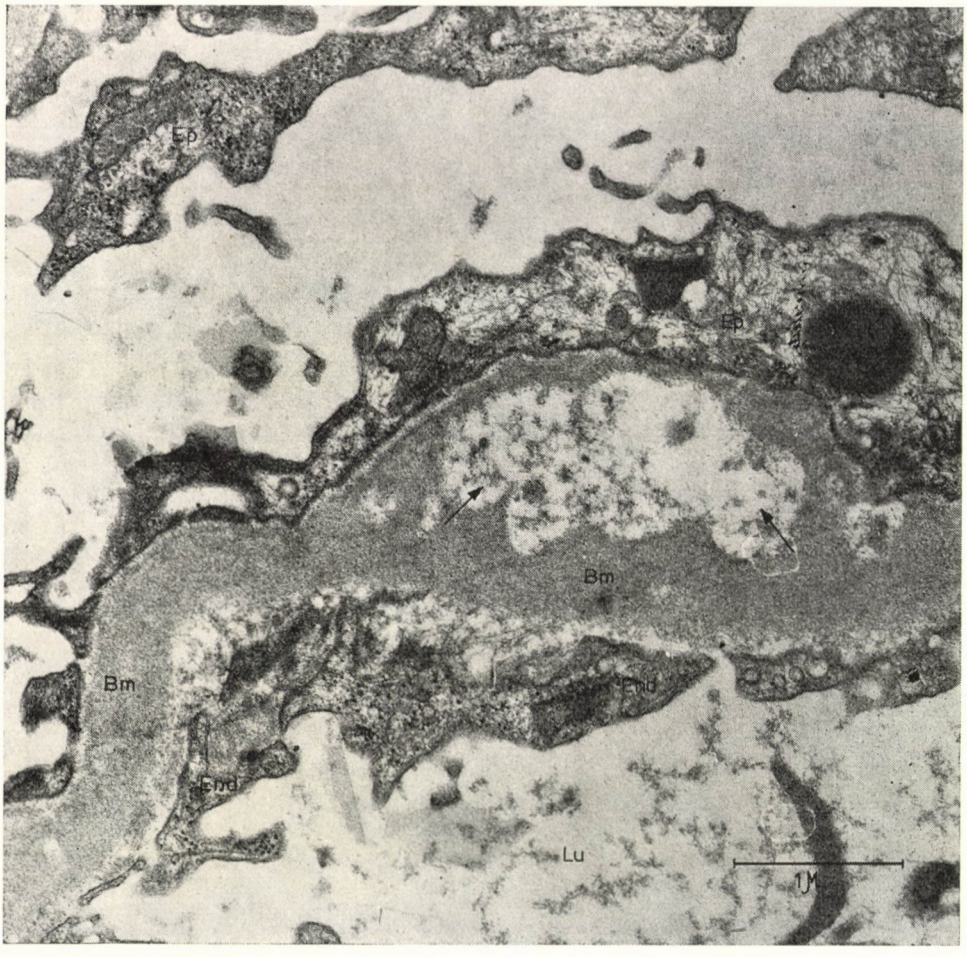

Fig. 9. Marked thickening with moth-eaten defect of glomerular basement membrane (arrow). (×30,000)

may be considered. First, immunosuppressive therapy may have affected the immune activity [23] or, else, the chronicity of the process may have been responsible for it. In order to judge this, we had only to check how many of the patients subjected to immunosuppressive therapy have become immunonegative or only slightly immunopositive, as opposed to those who had had no immunosuppressive treatment. In fact, 63 of the 70 patients had had immunosuppressive therapy. Repeated biopsy failed to reveal IgG or C' deposits in 16 cases; the deposits were confined to patchy areas in another 16; and were unchanged in 22 cases. No immunosuppressive therapy had been given in 7 cases. In 3 of

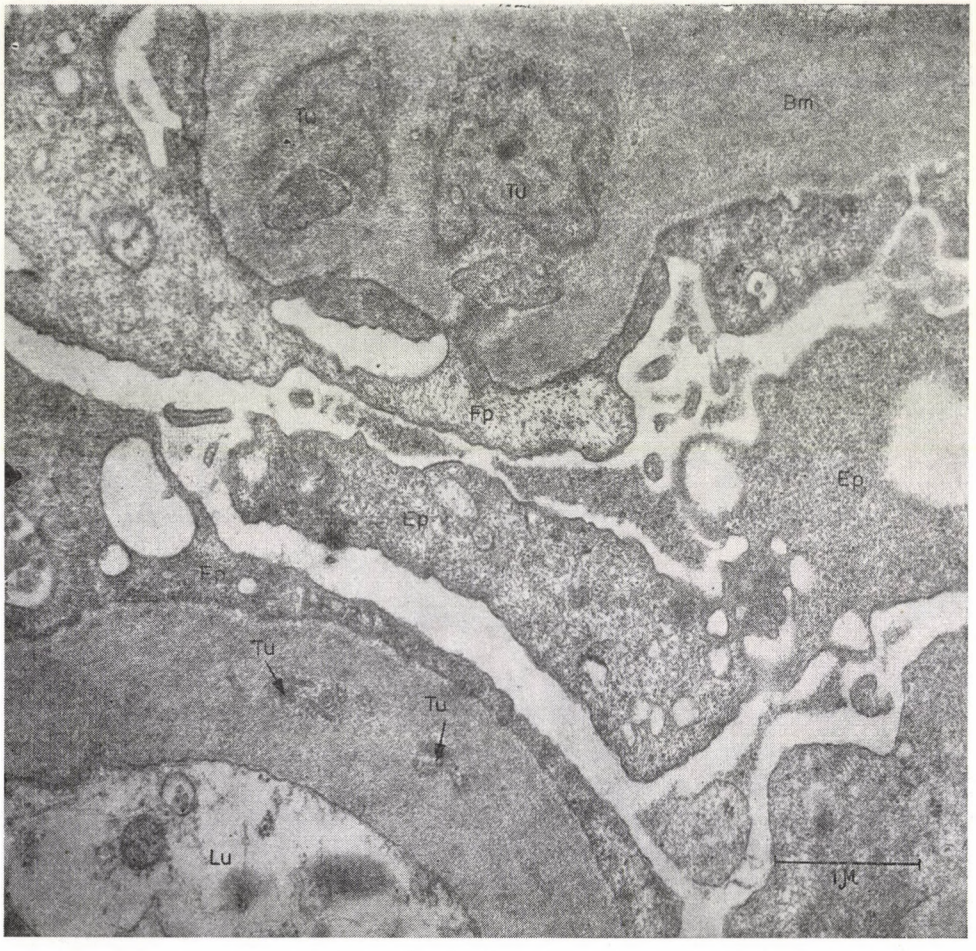

Fig. 10. Thickened glomerular basement membrane with tubular structures (arrow). Cytoplasmic vacuolisation of the epith elialcells and fusion of epithelial foot processes. (×26,220)

these, IgG and C' were no longer demonstrable on the basement membrane (spontaneous healing), in 3 the deposits had remained unchanged and in 1 they had slightly diminished. Though the number of patients with no immunosuppressive therapy was too small to be conclusive, the proportion of those in which immune activity remained unchanged was remarkably high. The other alternative, namely that of chronicity, could also be supposed in the cases of long-standing glomerulonephritis. However, consideration must be given to the fact that, despite of the chronicity of the process, the IgG and C' deposits on the glomerular basement membrane remained none the less unchanged in 25 cases.

The electron microscopic changes in the follow-up biopsy material were studied in 25 cases. The change most essential in comparison with the first biopsy was the presence of moth-eaten defects at numerous sites of the basement membrane, presumably at those of earlier deposits of electro-dense material. Actually, this material was still detectable at the periphery of the moth-eaten defects (Fig. 9). In addition, the same material, though indistinctly in some cases, was still observable at sites of the subepithelial or subendothelial aspect of the thickened basement membrane. Some foot processes of epithelial cells and also endothelial cells projected into the basement membrane, and in 3 cases tubular structure were recognizable on the thickened basement membrane (Fig. 10).

Discussion

The present findings indicate in accordance with published evidence that immunosuppressive therapy results in a decrease or disappearance of IgG and C'-deposits on the glomerular basmeent membrane and in some cases in the production of fibrin deposits.

The morphological features of renal disease have extensively been studied by repeated biopsies. MIESCHER and PARONETTO [19] noted a decrease in glomerular IgG in SLE patients on immunosuppressive therapy and considered the fibrin deposits to be due to changes in the clotting mechanism or to the vascular damage. CAMERON [3] stressed the significance of two adverse factors in the pathogenesis of glomerulonephritis, *viz.* complement and coagulation. In fact, coagulation leads to local tissue lesions as a result of microthromboses and inflammation. The mediator of inflammation is Factor XII (Hageman-factor) [20] which, on its part, may be activated by an antigen-antibody complex, among others, while both plasmin and thrombin by the complement. In this manner, coagulation activates the complement and gives rise to an inflammatory process. Moreover, the complement by some unexplained mechanism may initiate coagulation. In human proliferative and in crescent-forming glomerulonephritis as well as in certain forms of experimental glomerulonephritis, fibrin breakdown products are demonstrable in large amounts in the urine (CLARKSON et al., [5]). This seems to be due to a degradation in situ of the glomerular fibrin or fibrinogen deposite. CAMERON [3] ascribes a major part to chronic intravascular coagulation in the pathogenesis of human glomerulonephritis. This would agree with our observation according to which in glomerulonephritis with no tendency to healing glomerular fibrin deposits are demonstrable even in the absence of immunological activity. DODGE et al. [7] on the evidence of renal biopsy in children, reported on hypercellularity, mesangial deposits and "humps" of the basement membrane in cases with no tendency to regression 2 years after the onset of the process. The appearance of

epimembraneous "humps" would be a sign of progression of acute glomerulonephritis. In our own material there were, however, cases showing complete regression in spite of the presence of epimembraneous deposits. SEYMOUR et al. [23] found in SLE nephritis that the immune deposits may significantly change in amount, configuration and site in the course of the process. In the case of successful therapy they decrease in mass and assume a pale, flocculär appearance indicative of partial resolution. However, the deposits may remain unaffected by treatment and even increase or shift from subendothelial to intramembraneous or epimembraneous sites. In our cases of glomerulonephritis we, too, have observed a decrease and fading of the immune deposits, and in one case, the deposits were subendothelial at the first and subepithelial at the second, biopsy. Similar immunohistological findings have been reported by TAKEBAYASHI et al. [24] and LANGE et al. [17]. EHRENREICH and CHURG [8], on the evidence of electron microscopic studies of the successive stages of the process suggested that the bulgings of the basement membrane were due to subepithelial protein precipitates and that the subsequent diffuse thickening of the basement membrane ensued as a result of an incorporation of the precipitate. With the further advance of the process the precipitate may disappear from the basement membrane and leave moth-eaten defects, despite the persistence of the diffuse thickening of the basement membrane. On the evidence of other data [25, 18], in this form, too, there is a slight proliferation of mesangial cells. We could confirm in numerous instances the presence on the basement membrane of moth-eaten defects and of a faint precipitate. It has been pointed out by SEYMOUR et al. [23] that the humps of the basement membrane appear 4 to 6 weeks after onset, and that their number is related to the severity of the process. While in the view of these authors epimembraneous glomerulonephritis does not respond to therapy, ROSEN [21] noted regression even in these cases. Remission in children was reported by HABIB et al. [12], and in adults by HOPPER et al. [13]. KOBAYASHI [16] observed the transformation of what appeared in the first biopsy as focal glomerulonephritis into diffuse glomerulonephritis, confirmed by later repeated biopsy. We had a similar case, but in our other patient with focal glomerulonephritis, recovery was complete.

Renal biopsy allows to correlate the clinical symptoms and the morphological features. According to HEPTINSTALL [14], haematuria and proteinuria are often unrelated to the severity of the glomerular lesion. BERMAN et al. [1] and COHEN et al. [6] reported on histologically confirmed glomerulonephritis negative urinary findings. Conversely, glomerular changes may be absent or confined to focal glomerulonephritis in patients with massive haematuria. According to HEPTINSTALL [14] diffuse mesangial cell proliferation ("lobular stalks") is indicative of chronicity or of a progressive tendency of the process and may be associated with persistent proteinuria. Further morphological signs

of clinical progress include glomerular necrosis, fibrosis, capillary thrombosis, proliferation of Bowman's capsule, adherence of glomerular tufts, and the appearance of mesangial IgG and C' deposits. Our findings are in line with these observations, since morphological normalization ensued in some of our cases despite the persistence of positive urinary findings. This might be due to the fact that despite apparent morphological healing there were still some signs of the course of glomerulonephritis, such as mesangial hyperplasia and occasional inhomogeneous thickening of the basement membrane. On the other hand, in some other cases despite persistent glomerulonephritis the urine was negative.

Tubular structures have been identified in the cytoplasm of glomerular capillary endothelial cells in SLE nephropathy and also in glomerulonephritis. According to GYÖRKEY and SINKOVITS [10], HAAS and YUNIS [11], GARANCIS et al. [9], these structures represent myxoviruses. In the present material tubular structures were noted in the glomerular basement membrane in three cases, but these structures seemed more elongated than those described in the endothelial cells. Clarification of their nature requires further studies.

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EFFECT OF SERUM α_1-ANTITRYPSIN ON THE PROTEOLYTIC ACTIVITY OF PURULENT SPUTUM

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Inhibition of the proteolytic activity of purulent sputum by normal human serum and by sera of low α_1-antitrypsin concentration has been studied. Sera of low α_1-antitrypsin concentration exerted a poor inhibitory action; the degree of inhibition was in direct correlation with the α_1-antitrypsin level.

Ever since 1963, when the relationship between pulmonary emphysema and α_1-antitrypsin deficiency had been first pointed out by LAURELL and ERIKSSON [26] the subject has been extensively studied [3, 4, 6, 10, 16, 18, 19, 20, 36]. It has been confirmed that the deficiency is of a genetic nature and of autosomal co-dominant transmission, and the proportion of homozygotes (ZZ) in the normal population was estimated at 0.1% and that of heterozygotes (MZ) at 5% [16, 20, 36]. The mechanism accounting for the production of pulmonary emphysema in the individuals with inherited enzyme deficiency is believed to be due to uncontrolled digestion of pulmonary tissue by the proteolytic enzymes released from leukocytes and macrophages [17, 22, 23]. This is consistent with the observation that leukoproteases digest human pulmonary tissue and haemoglobin substrate in the same way and that human serum inhibits this activity on various substrates [22]. α_1-antitrypsin is the prevalent proteinase-inhibitor in human serum. At normal concentrations it accounts for approximately 90% of the total inhibitory capacity, the remaining 10% being shared between α_2-macroglobulin, α_2-antitrypsin, interalpha-trypsin-inhibitor and α_1-antichymotrypsin. The inhibitory activity of the last-named factors is, however, insignificant [9].

α_1-antitrypsin, as a non-specific proteolytic enzyme inhibitor, has been shown to inhibit pancreatic elastase [37, 39], as well as trypsin, chymotrypsin, plasmin, thrombin [33], proteases of bacterial or plant origin [21], kallikrein and leukocyte proteases [22, 23]. On the other hand, it does not affect pepsin [24]. These observations have prompted us to examine to what extent the proteolytic activity of purulent sputum was inhibited by normal serum and by serum with deficient antitrypsin levels.

Materials and Methods

Serial dilutions prepared from sera of various α_1-antitrypsin levels were studied for their inhibitory effect on labile protease activity of purulent sputum. Sputum was collected from subjects

a) having completed the British Medical Research Council questionnaire in respect of the history (long-standing cough, expectoration) in the affirmative;

b) with purulent sputum at the time of study.

The pathologic sera were obtained from 40 to 60 years old non-smoking females with non-specific chronic respiratory disease [36], the normal sera from an age-matched normal female population. The serum α_1-antitrypsin level was normal (0.80 to 1.2 mg/ml) in five, intermediary (0.50 to 0.60 mg/ml) in five, and deficient (0.20 to 0.40 mg/ml) in five cases. Total inhibitory capacity (TIC) was estimated by the method of ERIKSSON [3] on the basis of the inhibitory effect of serum on amidase activity of trypsin measured on a synthetic substrate. The serum dilutions were 1 : 8, 1 : 16 and 1 : 32.

One to two g aliquots of the sputum sample were weighed on an analytical balance, made up with distilled water to tenfold weight, mixed by means of a mixer for 30 to 60 min., and homogenized in portions in a Potter glass homogenizer. The volume of the agneous suspension of approximately 10% thus obtained was measured, made up with analytical grade NaCl to a concentration of 1 M, cooled to +2 to —4 °C and centrifuged at 16,000 r.p.m. for 15 min. The supernatant was poured off and processed immediately. Protease activity was estimated on native bovine haemoglobin as the substrate (2% bovine haemoglobin in 0.05 *M* phosphate buffer, pH = 7.5, with 1 : 50,000 merthiolate as preservative, stored at +4 °C until use).

The components were

	Control test	Inhibition test
Sputum	1.0 ml	1.0 ml
Serum	—	30—60—120 μl
Substrate	5.0 ml	5.0 ml

Physiological saline 120 μl.

The haemoglobin substrate and the sputum-serum and sputum-saline mixtures, were immersed in a water bath of 37 °C for 10 minutes. Then 5 ml of the haemoglobin solution was added to the above mixtures and mixed. Two ml aliquots were withdrawn from the control sample immediately and at 2 hours, from the other samples only at 2 hours, i.e. after incubation in a 37° C water bath. The portion withdrawn immediately served as the blank (zero point). To the 2 ml portion, 4.0 ml of 0.3 *M* trichloroacetic acid was added, the mixture was shaken and left to stand at room temperature for 30 min., and filtered. Five ml 0.5 *M* NaOH and 1.5 ml Folin's phenol reagent (1 : 3 dilution) were added to 2.5 ml of the filtrate and after standing for 5 min., the extinction was measured in 1 cm cuvettes against distilled water at 675 nm.

The amounts of serum used were 30, 60 and 120 μl.

From the 2 hour extinction reading the blank value was subtracted; the ratio of the extinction of the serum-free control sample and the serum-containing sample was expressed in per cents.

Results

Inhibition of labile leukoprotease by sera of various antitrypsin levels, expressed in per cents, is shown in Fig. 1. It is seen that normal sera, even at dilutions of 1 : 16, exerted an inhibitory effect of 84 to 90 per cent, on the

proteolytic activity of sputum, while sera from heterozygous subjects failed to inhibit at dilutions over 1 : 8. Sera of homozygous subjects revealed a decrease of inhibitory capacity (10 to 30%) at dilutions as high as 1 : 8.

The degree of inhibition was found to correlate directly with the α_1-antitrypsin level. The ability of individuals with homozygous and heterozygous enzyme deficiency to inhibit leukoprotease activity was correspondingly reduced (Fig. 1).

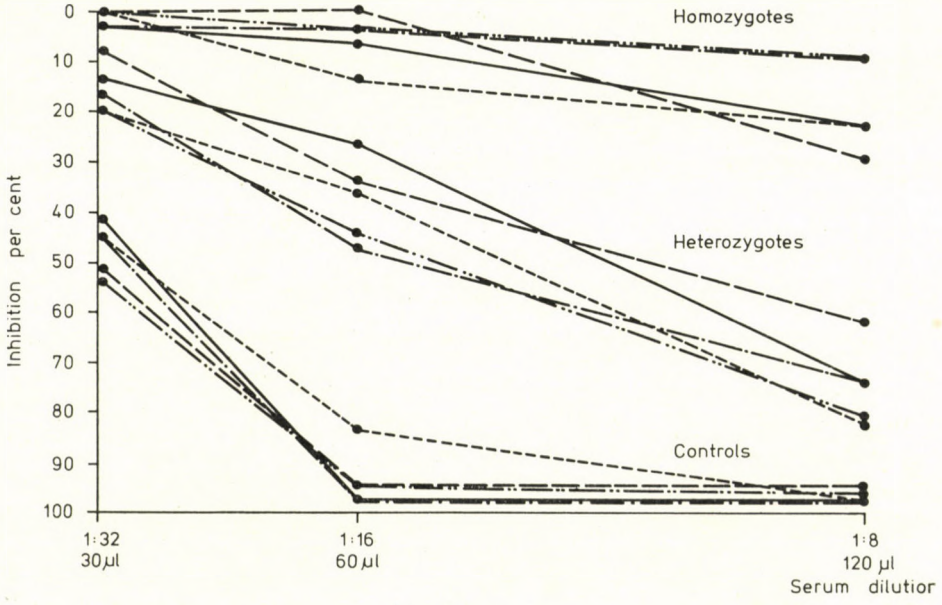

Fig. 1

Discussion

Purulent sputum contains two major leukoproteases, active at physiological pH-levels. The labile leukoprotease of trypsin character is denaturated by heating at 65 °C. The stable factor, leukocytic elastase, is resistant to temperatures of 65 °C. JANOFF and SCHERER [11, 12] identified an elastase-like enzyme in the granule fraction of human polymorphonuclear leukocytes. This factor has been found to affect the elastic fibers and the basement membrane, α_1-antitrypsin being inhibitory to its activity [13].

Proteolytic and elastolytic activities are absent from mucoid sputum and are unrelated to any particular infecting organism [22]. Both types of protease were shown to digest pulmonary tissue, the labile enzyme being more active than the stable one [22]. GRAHAM [8] and RINDERKNECHT [34] suggest the possibility of a synergism between the two enzymes.

The alveolar macrophages also contain proteolytic enzymes. Though the proteolytic activity of these enzymes amounts to 10% only of that of the leukocytic granules, their biological activity is greater, because they are less liable to inhibition. Tobacco smoke has been found to elicit an intensive macrophage activity [22]. McLAUGHLIN [28] identified pigment-laden macrophages in the vicinity of destructive lesions in smokers' lungs as well as the sputum of smokers. The brown pigment has been found similar to some components of tobacco smoke and was made responsible for the increase in number, activity and destructive potential of the macrophages of smokers' lung. FITZPATRICK and HOSPELHORN [5] and MANDL and KELLER [27] have shown a significant variation in the chemical composition of the elastic tissue in emphysema that the normal ratio between non polar and polar amino acids was shifted toward the polar amino acid. Elastin consists of an inner core of polar and of an outer shell of non-polar amino acids. The outer shell gives the fiber its elastic properties and the observed changes could be the result of proteolytic attack, and would account for the changes in the mechanical behaviour of the lung in pulmonary emphysema.

ADAMSON et al. [1] found that elastin content was decreased in the lungs of subjects with α_1-antitrypsin-deficiency and pulmonary emphysema. Elastolytic activity may thus play a primary role in the production of pulmonary emphysema [17, 37, 22], though the question whether the elastin or collagen contents of the lung are modified in pulmonary emphysema is still controversial [30, 31]. PECORA [32] found an increase of collagen in emphysematous lungs. ADAMSON [1] and JOHNSON and ANDREWS [14], and BRISCOE and LORING [2] observed an age-related increase in pulmonary elastin and a loss of pulmonary collagen, irrespective of the presence of emphysema.

MUSTAFA and CROSS [29], studying the biochemical effects of proteolytic enzymes at cellular and subcellular levels found that these enzymes inhibited the respiration of cell homogenisates and mitochondria, as also ATP hydrolysis. This would suggest that proteases may not only damage the connective tissue but also affect the integrity of the cellular and subcellular elements of other pulmonary structures.

These lesions of the pulmonary parenchyma produced by proteases of plant or bacterial origin are indicative of the potential role of these enzymes.

GOLDRING et al. [7] observed pulmonary lesions similar in morphology to human emphysema of the panacinar type after the intratracheal injection or aeorosol inhalation of papain, bromelain, ficin or bacterial proteases. LIEBERMANN [25] assumed that the plant-proteases acted merely by stimulation of leukocyte and macrophage accumulation, and it is from these cellular elements that the proteases of destructive action are released.

KIMBELL [15] observed pulmonary emphysema in dogs 8 days after exposure to aerosols of human and canine polymorphonuclear leukocyte

homogenisates. The lesions were less extensive if rabbit leukocytes or canine alveolar macrophages had been applied. Erythrocyte and monocyte homogenates were inactive in this respect.

The present findings, in accordance with data in the literature, thus lend support to the protease pathogenesis by linking up the state of α_1-antitrypsin deficiency with the production of pulmonary emphysema.

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RENAL VEIN NATIVE PLASMA PRESSOR ACTIVITY

By

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The diagnostic value of renal venous native plasma pressor activity has been studied by correlating its values with the findings of serial nephro-angiography and of renal venous plasma renin activity in hypertensive subjects. Although renal venous native plasma pressor activity was found to vary in proportion to the logarithm of renal venous plasma renin activity, the falsely positive results make it none the less unsuited for the replacement of the latter as a diagnostic test for the demonstration of a possible involvement of renal arterial stenosis in individual cases of hypertensive disease.

The primary significance of serial nephro-angiography in the clinical study of renovascular hypertension has universally been accepted. Opinions are divided on the choice of a procedure for the study of the functional involvements of renal arterial stenosis. The majority of authors applies the renal venous plasma renin activity (RV—PRA) assay in order to confirm or to reject the suspicion of renovascular hypertension, in other words, the necessity for surgical correction. On the other hand, MCPHAUL et al. [1, 2, 3] rely on measurements of renal venous native plasma pressor activity (RV—NPPA) for the clarification of relationships between renal arterial stenosis and arterial hypertension. In addition to its simplicity, the latter method allows to measure the resultant of the pressor and depressor activities in renal venous blood. Since, however, the reliability of this procedure has been questioned [4] we undertook acute experiments for the assessment of its value [5]. We found that a restriction of renal blood flow in dogs was followed by a significant increase in RV—NPPA, due to a quantitative increase of pressor substances rather than to increased concentration.

In the present study the diagnostic value of RV—NPPA has been examined in hypertensive subjects, comparing the results with those of serial nephro-angiography and of renal venous plasma renin activity.

Material and Method

Thirtysix patients with hypertensive disease were studied. The possibilities of pheochromocytoma, coarctation of aorta, nephritis, etc., having been ruled out, the subsequent investigations were directed at the differentiation between essential and renovascular hypertension. Mean systemic blood pressure (1/3 of systolic +2/3 of diastolic pressure) was above 100 mmHg in every patient, 17 of which had a diastolic pressure above 110 mmHg.

Plasma and urine values were studied by the conventional procedures.

Antihypertensive treatment was discontinued 7 days before the test. For the stimulation of pressor production, in the last three days a diet containing 10 mEq NaCl daily was given and hydrochlorothiazide was administered in doses of 50 mg daily. Serial nephro-angiography and sampling from both renal veins were performed by SELDINGER's method [6].

Renin activity was measured according to BOUCHER [7].

Estimation of RV-NPPA. The blood samples were collected in a recipient containing one drop of concentrated heparin which had been pre-cooled and stored among salted ice-cubes. The collected samples were then centrifuged at 4 °C for 10 min at 5,000 *g* in a cooled MSE centrifuge type MK2. Aliquots of 1 ml plasma were measured into pre-cooled flasks stored in the deep freezer at —20 °C until the day of bioassay when the deep-frozen samples were thawed at room-temperature and studied for pressor activity.

Bioassay. Home-bred male rats of 150 to 180 g body weight were subjected to bilateral nephrectomy under ether anaesthesia 18 to 24 hours before the assay. Then the fasted animals were anaesthetized with 1 g/kg urethan, and injected subcutaneously with 15 mg/kg of 0.5% chlorisondamine-chloride (Ecolid Ciba) or 2.5 mg/kg hexamethonium, in addition to 0.5 mg/kg of 0.05% atropine, made up to 1 ml with a 5% polyvinylpyrrolidone solution. 40 to 60 min later bilateral vagotomy was performed and the trachea was cannulated. Blood pressure was measured in the carotid artery with a mercury manometer and registered kymographically. A polyaethylene catheter was tied into the left femoral or jugular vein in the interest of rapid and reliable application of the test substances.

The plasma samples were administered in a constant volume of 0.5 ml with 0.1 ml Locke's solution. Measurements were made at intervals of 5 to 8 min. Every sample was titrated in accordance with the combinatorial rules on two rats and was administered to each animal on two occasions.

The bioassay is based on the principle that the unknown substance has to be administered in a concentration corresponding to the activity of the standard. To find the dose affording this concentration is a laborious procedure. The advantage of the present method lies, in addition to dispensing with biochemical preparation, in the possibility of measuring the activity of a given dose (0.5 ml) of the unknown substance (renal venous native plasma).

Evaluation. Native plasma pressor activity was estimated on the basis of the maximum hypertensive activity of the plasma samples, using as a standard 1, 2 or 4 ng angiotensin II (Hypertensin, Ciba).

For the assessment of RV-NPPA, various criteria have been elaborated. Earlier the RV-NPPA was considered pathologic if the hypertensive effect of 0.5 plasma was in excess of 10 mmHg (MCPHAUL et al. [1]). Later this was modified by GROLLMAN and EBIHARA [8] defining RV-NPPA as pathologic if the hypertensive effect of 1.0 ml plasma exceeded that of 5 ng of standard angiotensin II.

For the quantitative comparison of the samples the following scheme was adopted. Consideration being given to the differences in individual sensitivity of the animals, the hypertensive activity of the plasma samples was defined by the quotient of the peak hypertensive response in mmHg of the plasma sample and that of 1 ng angiotensin II. Evaluation was based on these quotients. RV-NPPA activity was considered abnormally high if the quotient was more than 1.9, in agreement with the criterium defined by GROLLMAN and EBIHARA [8]. According to retrospective analysis, localization is not possible unless RV-NPPA of one side is abnormally high and there is a difference of more than 0.5 between the RV-NPPA-quotients of the two sides.

RV-PRA was regarded as abnormally high if after provocation the value for the side exhibiting a higher activity exceeded the values of 1,000 ng angiotensin II/100 ml plasma/3 hr incubation. The ratio of the two sides was defined by the renin quotient, i.e. the quotient of the higher and the lower value. (In this manner the renin quotient was 1 or more.) The quotient between the values for the side of higher and for that of lower activity had to be in excess of 1.6 to be considered pathognomonic [9].

The result of serial nephro-angiography (SNAG) was regarded as pathologic if the renal artery was narrowed to 50% or less of its lumen [10] and if in the phase of arterial arborization the excretion of contrast material was protracted [9].

Biometry. For statistical analysis of the data, Student's two-sample *t* test or, in case of unequal within-group variances (*F* test), the *d* test was applied [11]. The correlations between the hypertensive effect and renin activity (RV-NPPA and RV-PRA) of the plasma samples were tested also by regression analysis.

Results

1. Renal venous native plasma pressor activity.

In order to test the diagnostic value of RV—NPPA, the data for the 36 patients were grouped according to Table I, representing the mean values and the standard error of the mean (Table I),

a) on the basis of RV—PRA;

b) on the basis of significant renal arterial stenosis;

c) in the case of a significant renal arterial stenosis, on the basis of RV—PRA.

The values were compared between subgroups *a)*, *b)* and *c)*.

Since by our standards no pathologically high renin values were found in the absence of morphological changes, the pathologic cases of group *a)* correspond to those of group *c)*, both forming a diagnostic subgroup of renovascular hypertension.

a) RV—PRA.

The values for RV—NPPA were significantly higher in the subgroup of increased renin activity than in that of normal renin activity. In the subgroup of pathologically high renin activity, RV—NPPA on the side of higher activity was 1.4 times, the difference between RV—NPPA of the sides of higher and of lower activity was 3.6-times, as high as in the subgroup of normal renin activity. At the same time, in the subgroup of increased renin activity, RV—PRA on the side of higher activity was 9 times, the renin-quotient twice, as high as in the normal subgroup. The difference reflected in the clinical data was confined to a biologically negligible difference in the specific gravity of spontaneous morning urine.

b) Renal arterial stenosis.

There was no deviation between the subgroup of significant renal arterial stenosis and the morphologically normal subgroup in respect of RV—NPPA on the side of higher activity. On the other hand, there was a minor difference in RV—NPPA between the sides of higher and of lower activities. In the case of renal arterial stenosis, RV—PRA as well as the renin-quotient on the side of higher activity were significantly above the values for patients with a normal serial nephro-angiogram. Clinically there was again an insignificant difference in the specific gravity of spontaneous morning urine.

c) RV—PRA in significant renal arterial stenosis.

The RV—NPPA values for the side of higher activity as well as the difference between the sides of higher and of lower activity revealed a significant deviation between the two subgroups, the difference for the positive subgroup i.e. that of renovascular hypertension, being three times that for the negative subgroup. In accordance with the grouping scheme on which the present analysis has been based, the RV—PRA of the sides of higher and of lower

Table I

Mean values and standard error of the mean

	a) Grouping on the basis of RV-PRA		*b)* Grouping on the basis of significant renal arterial stenosis		*c)* In the cases of significant renal arterial stenosis grouping on the basis of RV-PRA	
	positive	negative	positive	negative	positive	negative
n	6	30	14	22	6	8
Age	38.3 ± 2.3	33.1 ± 2.1	38.8 ± 2.2	31.8 ± 2.4	38.3 ± 2.3	38.0 ± 4.2
Mean arterial blood pressure, mmHg	145.2 ± 6.3	132.8 ± 4.8	137.7 ± 5.6	124.9 ± 4.3	145.2 ± 6.3	161.7 ± 9.5
Urine specific gravity	1020.2 ± 1.3	1024.1 ± 0.8*	1020.5 ± 1.0	1024.8 ± 0.9*	1020.2 ± 1.3	1021.3 ± 1.7
Ccr: ml/min	105.8 ± 11.7	112.8 ± 7.4	102.5 ± 7.8	114.2 ± 9.2	105.8 ± 11.7	107.7 ± 9.0
Serum creatinine, mg/100 ml	0.96 ± 0.07	0.99 ± 0.04	1.17 ± 0.14	0.95 ± 0.05	0.96 ± 0.07	1.12 ± 0.09
Serum Na^+, mEq/liter	139.0 ± 1.5	140.3 ± 0.56	139.3 ± 1.1	140.4 ± 0.5	139.0 ± 1.5	139.8 ± 1.9
Serum K^+, mEq/liter	4.03 ± 0.18	4.26 ± 0.07	4.29 ± 0.14	4.21 ± 0.07	4.03 ± 0.18	4.45 ± 0.18
Hb, g/100 ml	14.4 ± 0.2	13.5 ± 0.4	13.8 ± 0.5	13.6 ± 0.4	14.3 ± 0.2	13.4 ± 1.0
RV-PRA ng/100 ml side of higher activity	9867 ± 4008	1014 ± 156**	5386 ± 2344	1052 ± 187**	9867 ± 4008	905 ± 262**
RV-PRA ng/100 ml side of lower activity	4000 ± 2045	739 ± 122	2296 ± 1104	779 ± 151	4000 ± 2045	597 ± 160**
Higher/lower RV-PRA quotient	2.90 ± 0.41	1.42 ± 0.06**	2.06 ± 0.27	1.40 ± 0.07**	2.9 ± 0.41	1.43 ± 0.12*
RV-NPPA $\frac{\text{P plasma}}{\text{P 1 ng}}$ side of higher activity	2.93 ± 0.518	2.05 ± 0.175*	2.44 ± 0.259	2.04 ± 0.238	2.93 ± 0.518	2.05 ± 0.175*
RV-NPPA $\frac{\text{P plasma}}{\text{P 1 ng}}$ side of lower activity	2.10 ± 0.276	1.84 ± 0.177	1.91 ± 0.151	1.86 ± 0.241	2.10 ± 0.276	1.84 ± 0.177
Difference of higher and lower RV-NPPA	0.83 ± 0.33	0.23 ± 0.04*	0.49 ± 0.15	0.21 ± 0.05*	0.83 ± 0.33	0.28 ± 0.34**

Comparison of positive and negative subgroups
no symbol $p > 0.05$
* $p < 0.05$
** $p < 0.01$

activity as well as the renin quotient, differed in the two groups to approximately the same extent as in group *a)*.

2. Relationship between RV—NPPA and RV—PRA.

Abnormally high RV—NPPA values were found in 6 patients; in 3 of them the pathologic RV—NPPA values were associated with pathologic SNAG and RV—PRA. In one case the renal artery revealed a significant

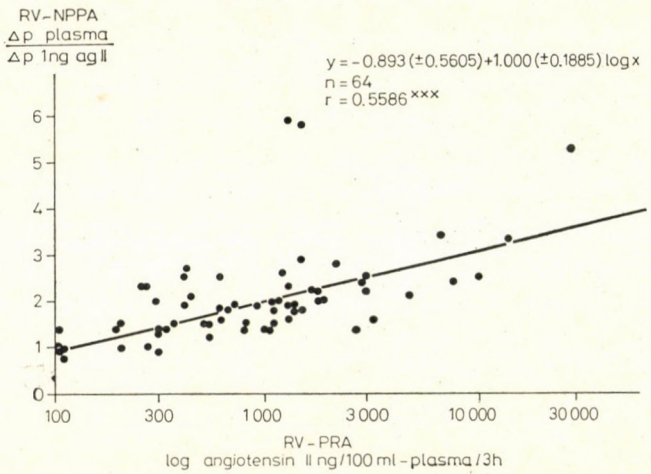

Fig. 1. Renal venous native plasma pressor activity (RV-NPPA) plotted against log renal venous plasma renin activity (RV-PRA). Each sample was estimated separately; statistical analysis was based on 64 paired data derived from 32 patients. The correlation between the paired data was highly significant ($p < 0.001$)

stenosis with a normal RV—PRA. In two cases the RV—NPPA had to be dismissed as falsely positive since neither the morphological study nor RV—PRA revealed any abnormality. These data might, however, reflect a difference between the results of RV—NPPA and of RV—PRA estimations.

RV—NPPA and RV—PRA of the individual kidney were compared on the basis of the overall data with the aid of correlation and regression analysis (Fig. 1). A highly significant correlation was found between RV—NPPA and the logarithm of RV—PRA, RV—NPPA increasing in proportion to the logarithm of RV—PRA.

Discussion

In the interest of an adequate evaluation of RV—NPPA, the patients were grouped from different points of view, as seen in Table I. No major difference could, however, be found between the individual groups in respect of age, blood pressure, clearance, urine and plasma constituents. Where both serial nephro-angiography and RV—PRA were pathologic, mean RV—NPPA values for the respective groups were likewise pathologic.

On comparing the overall results, a significant correlation between RV—NPPA and log RV—PRA was found (Fig. 1). GROLLMAN and EBIHARA [8] noted abnormally high RV—PRA and RV—NPPA values in two thirds of their cases.

On the evidence of the literature it seems no longer questionable that a pathologic SNAG associated with a pathologically high RV—PRA is conclusive of renovascular hypertension and thus an indication for surgery. In the present material no increased RV—PRA was found in the absence of morphological abnormalities. On the other hand, there were two cases with abnormally high, falsely positive RV—NPPA in the presence of normal SNAG and normal RV—PRA.

Estimation of RV—NPPA is considered fully informative and reliable only by MCPHAUL et al. [1, 3] and even these authors found it necessary to narrow down their criteria of evaluation [8]. The present findings showed in accordance with THOMPSON [4] that measurement of RV—NPPA, simple as it may be, offers no substite for the estimation of RV—PRA.

According to recent evidence [12], RV—NPPA is closely related to the amount of angiotensin I bound to an α_2-globulin in the venous blood of the ischaemic kidney. The advantage of radioimmunoassay is that it suits itself also for the measurement of angiotensin I.

*

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INHIBITION OF MOUSE SPLEEN CELL ROSETTE FORMATION BY COBALT IRRADIATION: MEASUREMENT OF THE IMMUNOSUPPRESSIVE EFFECT OF GAMMA-RADIATION

By

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Total body cobalt irradiation of Balb/c mice with a dose of $1/5LD_{50}$ proved to inhibit immune rosette formation. The irradiation reduced the number of rosette-forming cells and suppressed the rosette-forming capacity of the remaining lymphocyte population. T lymphocytes were particularly sensitive to the irradiation.

When irradiation was given before the antigen or on the day of its administration, suppression of rosette formation was more marked than with irradiation following the antigenic stimulus.

The results provide additional information on the immunocyte-suppressive activity of ionizing radiation.

Lymphocytes of mice immunized with heterologous erythrocytes are binding the red cells in the form of a rosette [8, 9, 26, 37, 38]. Rosette formation reflects one of the essential specific functions of the immunocompetent lymphocytes, i.e. their antigen-binding capacity [7]. The lymphocytes form the rosette by means of specific receptors [17, 19, 32] which are present on the surfaces of T and B lymphocytes, therefore the rosette phenomenon lends itself to the study of both lymphocyte populations.

The lymphocyte receptors being most sensitive to immunosuppressive factors, the rosette phenomenon to which intact receptors are essential, is eminently suitable for the measurement of the immunosuppressive activity of various drugs [1—4].

Inhibition of rosette formation by ionizing radiation has received little attention. It has been reported that irradiation of spleen cells in vitro is inhibitory to rosette formation [27], but evidence in vivo is lacking.

In previous studies the inhibition of rosette in vivo was found to be more informative for the immunosuppressive effect of drugs than such tests in vitro [20—22]. It was therefore expected to gain closer insight into the immunosuppressive effect of radiation by studying its effect on rosette formation in vivo. For irradiation, cobalt was selected because of the facility of its dosage and the homogeneity of its radiation.

Material and Method

1. Immunization and irradiation of mice.

Inbred two months old male Balb/c mice were injected intraperitoneally with 3×10^8 sheep red cells (SRC) on one occasion. Seven groups of 16 animals each were set up. Total body cobalt irradiation of 150 r (46 R/min) was given to three groups prior to immunization (at −10, −6 and −3 days, respectively), to one group on the day of immunization, and to three groups after immunization (at 1, 2 and 4 days, respectively).

2. The rosette test and its evaluation.

The dynamics of rosette formation being taken into consideration, the tests were performed on the 4th and the 8th day after immunization.

The capsule of the spleen was split lengthwise, the pulp was squeezed out, treated in Potter-cell homogenizer. The suspension was taken up in Parker's 199 medium containing 10% calf serum, aspirated through a 18 bore injection cannula for the elimination of stromal residues, and after two washings the cell counts were adjusted to 2×10^7/ml. Aliquots of 0.5 ml of each sample were incubated in 20/μg/ml azathioprine and similar aliquots in the medium, at 37 °C for 90 min. During this time blocking of the T-cell receptors by azathioprine is achieved [5]. After incubation the suspensions were washed and adjusted to the original cell count. Then 5×10^8/ml sheep red cells were added to each sample in the same volume. The suspensions were centrifuged at 200 *g* at 37 °C for 15 min, resuspended, and the number of rosettes per thousand lymphocytes was counted on a slide. In addition, the number of rosettes for the whole spleen was estimated on the basis of the spleen cell count.

The lymphocytes attaching a minimum of 5 SRC were regarded as rosette-forming cells (RFC).

In the azathioprine-treated samples, only the azathioprine-resistant B-lymphocytes, in the untreated samples both the T and B lymphocytes were forming rosettes. The difference between the two values gives the number of T-cell rosettes.

Rosette inhibition by immunosuppressive agents (in the present case by cobalt irradiation) was not considered significant unless the number of RFC was reduced by at least 25% [5].

Results

Fig. 1 demonstrates the reduction in the spleen cell count under the effect of irradiation. A reduction occurred in every group. It was most marked when irradiation was given close to or simultaneously with the antigenic

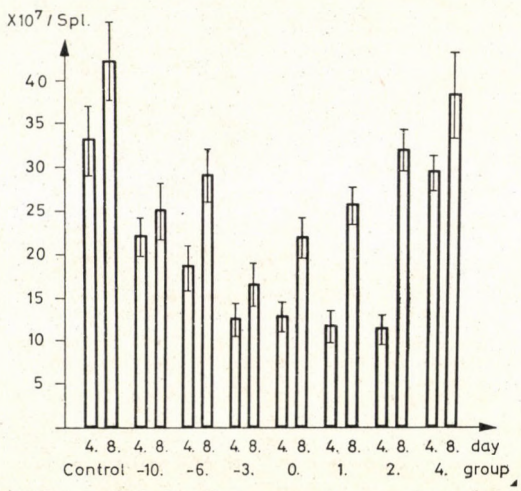

Fig. 1. Effect of Co-irradiation on the number of spleen cells

stimulus. The decrease in spleen cell count was more significant on the 4th than on the 8th day after immunization.

Fig. 2 illustrates the changes of the number of RFC after Co-irradiation.

The hatched portions of the columns represent the azathioprine-resistant, the empty portions the azathioprine-sensitive rosettes.

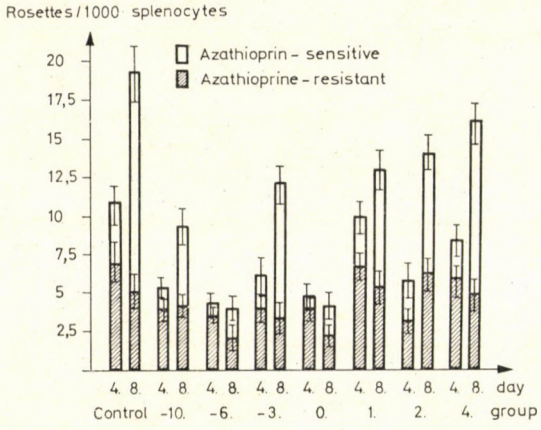

Fig. 2. Effect of Co-irradiation on the proportion of rosette-forming T and B lymphocytes

Irradiation administered prior to, or simultaneously with the antigenic stimulus resulted in a more marked rosette inhibition than did irradiation subsequent to immunization. This was demonstrable at the 4-day and 8-day readings alike.

While on the 4th day after immunization the RFC were prevalently azathioprine-resistant B lymphocytes, by the 8th day the rosettes formed by azathioprine-sensitive T lymphocytes had gained preponderance. In all the groups, the T cells were primarily affected.

Reduction in the number of RFC referred to the whole spleen is seen in Fig. 3.

The number of RFC referred to the total spleen can be calculated on the basis of the total number of spleen cells and of the proportion of RFC. The figures thus obtained reflect the complexity of the rosette-suppressive effect resulting from two factors, a reduction of the rosette-forming spleen-cell population, and a blocking of the rosette-forming capacity of the remaining lymphocytes.

The hatched portions of the columns represent the number of azathioprine-resistant, the empty portions that of the azathioprine-sensitive rosette-forming lymphocytes.

Readings taken at 4 and 8 days after immunization showed that the decrease in the rosette-forming cell population was more marked if the irra-

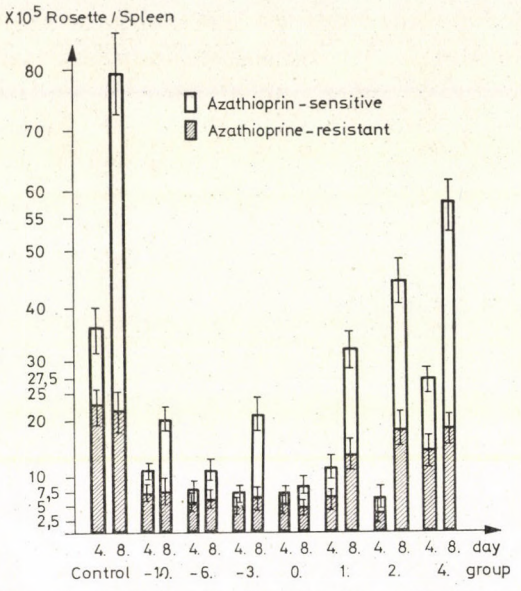

Fig. 3. Number of rosette-forming lymphocytes referred to total spleen

diation occurred a few days before or on the day of immunization instead of its application after the antigenic stimulus. Further, Co-irradiation primarily affected the T lymphocytes by reducing their population and by interfering with the rosette-forming capacity of the remaining cells.

Discussion

It has long been known that immunosuppression belongs to the biological effects of ionizing radiation, as reflected by the suppression of antibody production [17, 30, 32], by the prolongation of skin homograft survival [10, 13, 14] and by inhibition of skin reactions of tuberculin type [23, 25, 33, 35].

In addition to these tests, other procedures of more recent origin, based on lymphoblast transformation, inhibition of lymphocyte migration, lymphocytotoxicity in vitro, rosette formation etc., allow to estimate the immunosuppressive effect at the cellular level [29, 36, 20—22, 31]. Of these procedures, the lymphoblast transformation test has already been used for the measurement of the immunosuppressive effect of ionizing radiation [24], but the rosette inhibition test in vivo has not yet been employed for this purpose despite its high sensitivity to immunosuppressive factors.

It has been demonstrated by the present study that Co-irradiation affects the immunocytes. The cells of T and B types are of different radio-

sensitivity. Co-irradiation interferes in the first place with the rosette-forming capacity of the T lymphocytes. This implicates that the immunosuppressive effect of gamma-radiation primarily affects the cell-mediated immune reactions. This observation is in line with the data in the literature concerning the high resistance of antibody-forming cells to ionizing radiation [15, 28, 34]

As already mentioned, OSOBA [27] found that irradiation in vitro inhibited the rosette formation by spleen cells. The rosette inhibition test in vitro is, however, not conclusive enough to be accepted as a reliable indicator of immunosuppression, not only because the rosette-suppressive activity of a drug is not necessarily associated with immunosuppressive properties [29] but also because cellular cooperation and the various repair mechanisms fail to assert themselves in vitro.

In our experience, the inhibition of rosette formation in vivo by cytostatic agents, corticosteroids, antilymphocyte globulin preparations may be regarded as predictive of the chances of human immunosuppressive therapy [20—22]. The fact that Co-irradiation is inhibitory on rosette-formation might allow to assess by this method the immunosuppressive capacity of individual radiation doses. This would be of high practical interest, since the immunosuppressive properties of radiation are already utilized for homograft protection [10] as also for the suppression of inflammatory processes of autoimmune origin [31].

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HUMORAL IMMUNITY IN CHRONIC LIVER DISEASE

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The humoral immune response has been studied in various types of chronic liver disease (chronic active hepatitis, chronic persistent hepatitis, cirrhosis of liver, fatty liver) in 69 patients on the basis of the gamma-globulin level, sedimentation rate, thymol turbidity test, serum levels of the three main immunoglobulins, antibacterial antibody titres and factors of autoantibody character.

An enhanced humoral immune response was found to represent a common feature of chronic liver disease associated with mesenchymal reaction. The data referred to above, in particular the immunoglobulin and antibody levels, proved indicative of the presence or of the absence of a mesenchymal reaction. High antibody levels, especially for enteral bacteria, were found typical of chronic liver disease associated with a mesenchymal reaction, in opposition to non-enteral bacterial antibodies which remained practically unaffected. The presence of factors of auto-antibody nature points to a probable involvement of autoimmune mechanisms.

Extensive experimental and clinical studies in the last two decades showed that autoimmune factors may be involved in the pathomechanism of chronic liver disease or even account for its pathogenesis [2, 4, 8—13, 16—18, 21, 24, 25, 28—33, 36, 37].

Hypergammaglobulinaemia is a common feature of chronic liver disease [1, 22, 27, 44]. Its development has been attributed to auto-antibodies suggestive of an autoimmune mechanism on the one hand [12, 13, 28—30, 32, 33, 46], and to antibodies induced by exogenous antigens, on the other [6, 18, 19, 42, 43]. In this paper we shall report on studies of the humoral immune status in chronic liver disease.

Material and Method

The 69 patients forming the present material were divided on the basis of the clinical features, biochemical parameters and liver biopsy into the following groups

Chronic active hepatitis	20 cases
Chronic persistent hepatitis	13 ,,
Cirrhosis of the liver	23 ,,
Fatty liver	13 ,,

For the assessment of the humoral immune system the following studies were carried out.

1. Serum-proteins: gamma-globulin fraction (paper electrophoresis), red blood cell sedimentation rate (Westergreen), thymol turbidity test.

2. Quantitative immunoglobulin estimation by the radial immune diffusion procedure of MANCINI et al. [26];

3. Estimation of antibacterial antibody level by TAKÁTSY's micromethod [40] and the microhaemagglutination and haemolysis-inhibition procedures of BACKHAUSZ et al. [3];

4. Identification of factors of autoantibody nature, by procedures described earlier [35];

— study of antigammaglobulin rheumatoid factor by the Rose-Waaler and latex-reactions;

— indirect immunofluorescence according to WELLER—COONS [45] for the study of antinuclear factor (ANF) in calf thymus smears, and for anticytoplasm (antimitochondrial) antibody (ACA), in rat kidney sections;

— autoimmune complement fixation for the study of antibodies to subcellular fractions of homologous liver cells (nucleus, mitochondria, microsomes, supernatant);

— study of the LE-factor by means of the latex-nucleoprotein reagent of HYLAND.

The mean titre of normal antibacterial antibodies was estimated in the sera of 114, the serum immunoglobulins in 60 healthy adults. To these figures were referred the values of the patients [34]. The results of the immunoserological tests were checked with those obtained in 20 healthy young subjects [35]. For comparative analysis, Student's two-sample t-test, for that of the antibody titres as well as for the other laboratory results, the χ^2-test was used.

Results

The parameters regarded as indicative of serum protein changes are presented in Fig. 1. The difference is obvious between the chronic active hepatitis and fatty liver groups. Pathologically high values for gamma-globulin, erythrocyte sedimentation rate and thymol turbidity were significantly more frequent in the patients with chronic active hepatitis than in those with fatty liver ($p < 0.001$ for gamma-globulin; $p < 0.01$ for BSR and thymol turbidity). The difference between chronic persistent hepatitis and fatty liver was not significant ($p > 0.05$). In cirrhosis of the liver, compared with fatty liver, only the increase in the gamma-globulin level showed a significant difference ($p < 0.05$), in opposition to the two other parameters the distribution of which was similar in the two groups ($p > 0.05$).

The serum levels of the three main immunoglobulins are shown in Fig. 2. The individual immunoglobulin values usually exceeded the normal mean. In the individual groups, the difference was obvious between chronic active hepatitis and fatty liver. While in the former group the serum levels of all three immunoglobulins were significantly increased ($p < 0.001$), in the latter no significant deviation from the normal values was demonstrable ($p > 0.05$).

In persistent hepatitis, the mean serum-IgA and IgM levels ($p < 0.05$ for both values), in cirrhosis of the liver, the serum IgG and IgA levels ($p < 0.01$ for both values) were significantly increased.

Fig. 3 shows the antibacterial antibody titers (Fig. 3). Though there was a difference between chronic active hepatitis and fatty liver, this was less obvious than with the immunoglobulin values. The incidence of increased salmonella-antibody titers was significantly higher in chronic active and chronic persistent hepatitis than in fatty liver ($p < 0.01$ for both groups). In cirrhosis and in fatty liver, the salmonella antibody titre showed the same distribution ($p > 0.05$).

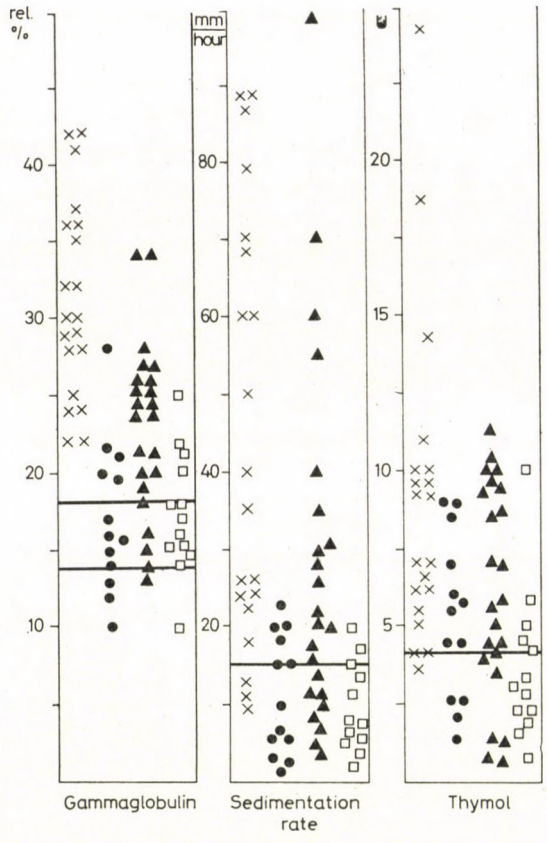

Fig. 1. Serum protein parameters

× Chronic active hepatitis
● Chronic persistent hepatitis
▲ Cirrhosis of liver
□ Fatty liver

The horizontal lines represent the limits of normal range

In the case of staphylo-alpha-antitoxin, no increase in antibody titre indicative of enhanced immune activity has been found. In chronic hepatic disease the incidence of an increased salmonella-antibody titre was significantly higher than that of increased staphylo-alpha-antibody titres ($p < 0.001$).

Fig. 4 showing the shigella-antibody titres, illustrates the tendency to increased levels. The titre was higher than normal in significantly more cases than the titre of staphylo-alpha-antitoxin ($p < 0.001$). The frequency of increased shigella-antibody-titres was significantly higher in chronic active and persistent hepatitis than in fatty liver ($p < 0.05$ for chronic active, $p < 0.001$ for chronic persistent hepatitis). In cirrhosis and in fatty liver the shigella-antibody titres displayed similar distribution ($p > 0.05$).

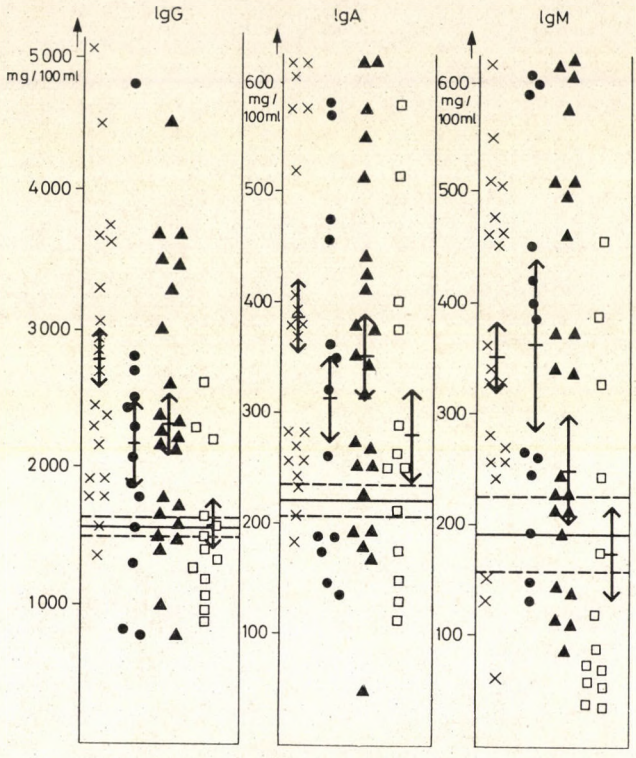

Fig. 2. Serum-immunoglobulin levels
The horizontal lines represent the normal mean ±SE, the perpendicular arrows the mean ±SE for the individual groups

Fig. 5 shows the E. coli antibody titres. The frequency of increased titres was significantly higher for these antibodies too than for staphylo-alpha-antibodies ($p < 0.001$). The number of patients with increased E. coli-antibody levels was significantly higher in the chronic active hepatitis ($p < 0.001$) and the cirrhosis ($p < 0.05$) groups than in the fatty liver group.

The frequency of factors of antibody character is shown in Fig. 6. In the group of chronic active hepatitis there were more RF-positive cases than in the other three groups together ($p < 0.05$). Immunofluorescence and complement fixation reactions were found to be far more specific than the RF. In the group of chronic active hepatitis both tests were positive in 18 out of 20 cases. The results of immunofluorescence were negative all throughout in fatty liver, those of autoimmune complement fixation in fatty liver and in persistent hepatitis. In chronic active hepatitis the two tests yielded far more positive results than in the three other groups together ($p < 0.001$).

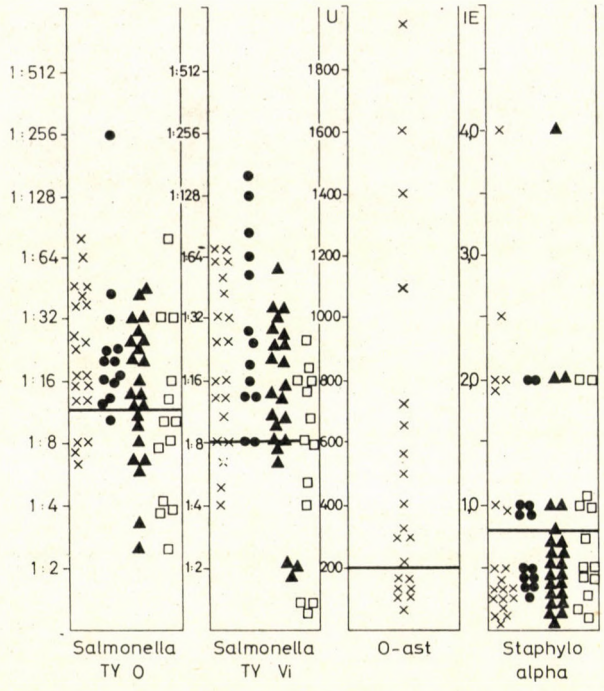

Fig. 3. Salmonella-, O-AST, staphylo-alpha antibody titres
Horizontal lines: normal mean values

Discussion

Dysproteinaemia is common in chronic liver disease, as reflected by the positivity of the colloid lability tests, the increased BSR, the high gamma-globulin and immunoglobulin levels and the high antibacterial antibody titres [1, 19, 22, 27, 44].

Dysproteinaemia of this kind may be attributed in the first place to a hyperfunction of the humoral immune system. This hyperfunction is closely connected with the extent of periportal lympho-plasmacellular infiltration, reflecting the degree of hepatic mesenchymal reaction [29], characteristic of the types of hepatic disease. The reaction is most typical in chronic active hepatitis and the least so in fatty liver. The parameters under study thus reflect a sharp difference between the group of chronic active hepatitis and of fatty liver. This is not always valid for individual cases, in all probability because there may be occasional cases of fatty liver with signs of mesenchymal reaction.

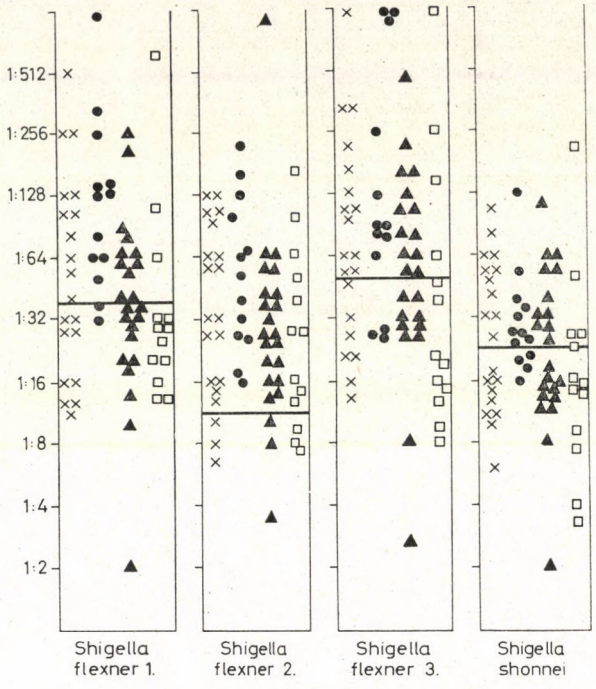

Fig. 4. Shigella antibody titres
Horizontal lines: normal mean values

In the groups of persistent hepatitis and of cirrhosis of the liver the parameters studied yielded normal as well as pathological values, owing to differences in the mesenchymal reaction accompanying these forms of chronic liver disease.

The changes of serum immunoglobulins regarded as typical of the individual types of chronic liver disease will not be discussed here, as our results were largely consistent with data in the literature [7, 14, 15, 23, 38, 41]. It must, however, be noted that no diagnostic conclusions have been drawn from the immunglobulin findings in individual cases [29].

The antibacterial antibody titres did not seem to reflect the changes in the serum immunoglobulin levels. This may have a dual explanation. First, the antibacterial antibody titres studied are unlikely to follow closely the serum immunoglobulin levels which represent in fact the totality of numerous serum antibody factors, although the enteral antibacterial antibodies correlate surprisingly well with the serum immunoglobulins. Second, the lack of a correlation of the titres of certain antibacterial antibodies with the serum immunoglobulin levels should not be dismissed as fortuitous. In particular, the absence

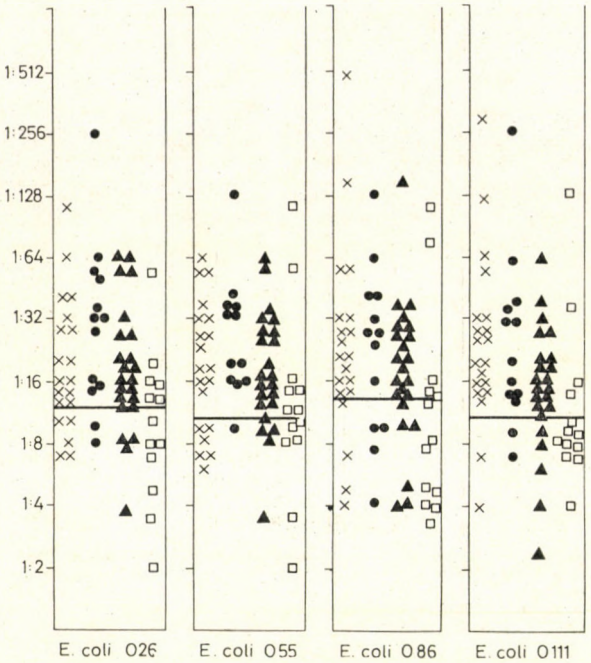

Fig. 5. E. coli antibody titres
Horizontal lines: normal mean values

of high titres indicative of an enhanced activity in case of staphylo-alpha antitoxin gives some food for thought. In fact, the prevalence of increased immunoglobulin and antibody levels in chronic liver disease has been connected to enhanced antigenic stimuli by enteral bacteria, i.e. to an impaired hepatic elimination of these antigens [5, 6, 42, 43]. The antibodies to enteral bacteria (in the present case to salmonella, shigella, E. coli) are certainly more consistent indicators of an enhanced immune activity than are the non-enteral staphylo-alpha antibodies. This is, however, not entirely valid for O-streptolysin antibody, a finding which seems to be at variance with the view referred to above. HAVENS [18] noted an enhanced humoral immune activity in response to active immunization with bacterial antigens in chronic liver disease despite the non-enteral origin of the antigens [18, 19]. It may be of interest to mention in this context that the most consistent rise in enteral antibody titre was found in persistent hepatitis, where reversibility, i.e. complete restitution, is common.

An increased humoral immune responsiveness may thus be regarded, on the evidence of the present results, as a general feature of chronic liver disease associated with a mesenchymal reaction. The studied parameters, in particular the immunoglobulin and antibody levels, though being indicative of the pre-

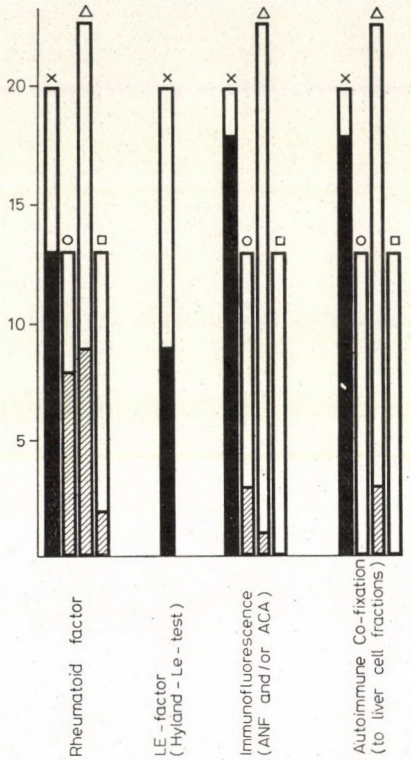

Fig. 6. Serum factors of autoantibody character
The columns represent the number of cases; their shaded portions, the positive cases

sence or absence of a mesenchymal reaction, offer no adequate basis for diagnosis in individual cases.

Demonstration of serum factors of autoantibody nature may be helpful in the detection of autoimmune patomechanisms [10, 23, 28—30, 33, 46], in particular, as in the present study, in the differentiation of chronic active hepatitis from other forms of chronic liver disease. Obviously, none of the existing procedures are exempt of false-negative or false-positive results [28, 30, 31].

Diagnosis of chronic liver disease is a complex task requiring clinical, biochemical, histological and immunological data. Closer insight into the pathogenesis, improvement of diagnostic possibilities, proper evaluation of the therapeutic factors, are objectives stimulating research for new tools of investigation. It might be rewarding to assess the data reflecting the status of cell-mediated immunity. Studies concerned with this question are in progress.

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EFFECT OF BETA-ADRENERGIC STIMULATION ON CORONARY BLOOD FLOW IN SEGMENTAL MYOCARDIAL ISCHAEMIA

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The coronary haemodynamic response to the administration 0.1 $\mu gkg^{-1}min^{-1}$ to 1.6 $\mu gkg^{-1}min^{-1}$ of isoprenaline was studied in anaesthetized open-chest dogs with normal coronary flow and in myocardial ischaemia induced by ligation of the anterior descending branch of the left coronary artery. Coronary blood flow was studied by the direct measurement of coronary sinus flow. Local myocardial blood flow of the ventricular area supplied by the descending coronary branch was studied by the heat clearance method. Though a slight decrease in the overall beta-adrenergic dilator response of the coronary system was found after occlusion of the descending coronary branch, no significant change in the slope of the dose-response curves of coronary dilatation was demonstrable. Isoprenaline was found to induce local vasodilatation in the ischaemic area too, which was of the order of 50% of that recorded prior to occlusion of the descending coronary branch. It is concluded that the collateral vasculature responds to beta-adrenergic impulses similarly as the coronary system.

Beta-adrenergic stimulation is well known to result in a marked increase in coronary blood flow, in association with an increased ventricular contractility and tachycardia. We ignore, however, whether after occlusion of a coronary branch the collaterals supplying the ischaemic myocardial area are also capable of dilatation. It is also uncertain whether the abnormal conditions resulting from coronary occlusion are apt to modify the responsiveness of the coronary adrenergic stimulation produced by the intravenous infusion of isoprenaline. By using this drug no simultaneous alpha-adrenergic stimulation of coronary vessels had to be taken into account, an event which would have still further added to the complexity of haemodynamic conditions prevailing in the ischaemic myocardial area.

Material and Method

10 dogs of either sex weighing between 14 to 22 kg were used. Anaesthesia was induced with 0.11 g/kg of chloralose. The trachea was cannulated for positive-pressure respiration with room air, at a peak pressure of 10 cm H_2O. The chest was opened in the fourth intercostal spaces. Thirty min before the measurements the animals were infused with 15 to 20 ml/kg dextran (Rheomacrodex). Bilateral cervical vagotomy was performed for the elimination of interfering reflex activity. Heparin served as anticoagulant; its first dose was 500 IU/kg body weight; the second, administered 90 min later, 250 IU/kg body weight.

Coronary flow was measured by two parallel procedures.

a) For measurement of venous outflow from the coronary sinus, a Morawitz cannula 6 mm in diameter provided with an inflatable balloon was inserted through the right auricular appendage into the sinus, and the venous coronary blood was returned via a wide poly-

ethylene cannula introduced through the central stump of the right jugular vein into the right atrium. A catheter connected with the T-branch of the cannula was fixed at a level corresponding to the right atrial pressure. It was opened for periods of 15 sec duration in order to measure the effluent coronary sinus blood, using a graduated cylinder and a stop-watch. The collected blood was then immediately returned to the animal. The pressure gradient prevailing in the entire artificial circuit was not higher than 3 mmHg even at peak blood flow.

b) The heat clearance method was applied for studying myocardial flow in the area supplied by the anterior descending coronary branch. The atraumatic, heated, flexible copper-constantan thermal probe was inserted into the myocardium at a distance from the major coronary branches and fixed with an epicardial stitch distal to the site of measurement. Heating was provided by stabilized direct current and an initial temperature difference of 1.5 °C was established between the heated and the reference poles of the probe. Then the temperature difference between the poles varying in inverse proportion to the flow was continuously recorded on a compensograph of high sensitivity (Kipp-Zonen micrograph). Tissue blood flow was expressed in per cents of 100% being the resting value found at the beginning of the experiment. The zero point of the tracing was represented by the thermal conductivity measured after having arrested the heart of the exsanguinated animal by an electric shock.

Myocardial ischaemia was induced by occlusion of the anterior descending coronary branch by means of a loop placed around it and pulled through a plastic tube. Occlusion was effected at a level between the upper and middle third of the artery over the point where it gives off a major branch running in oblique direction to the left ventricular margin. On the evidence of our earlier findings (URBANICS and JUHÁSZ-NAGY 1971), the ischaemia thus produced involves approximately 20% of the left ventricular musculature. The thermocouple was placed in every case within the ischaemic area which after the production of ischaemia was certain to receive its blood supply exclusively from the collaterals. In order to study collateral blood flow in a range as wide as possible, in individual experiments we varied the distance of the thermal probe from the presumable centre of the ischaemic zone.

Arterial blood pressure was measured via a catheter passed through the carotid into the ascending aorta by means of a Statham electromanometer and was registered on an Elema-Mingograph. Mean systemic blood pressure was determined by electric integration.

The coronary response was partly defined by the variations of blood flow partly by the values of vascular conductance calculated according to the formula,

$$\text{Conductance (per cent)} = \frac{Q \times \bar{p}_c}{Q_c \times \bar{p}} \times 100$$

where Q and p represent actual blood flow and mean systemic blood pressure, respectively, and Q_c and p_c the respective control values. pO_2 and pH were measured in serial arterial and coronary sinus blood samples with an Astrup apparatus in five successive experiments.

Dose-response curves of isoprenaline were determined by continuous infusion of the drug, modifying its doses in a geometric proportion in the range between 0.1 $\mu gkg^{-1}min^{-1}$ and 1.6 $\mu gkg^{-1}min^{-1}$. After equilibration of the effects of increasing doses ensuing in 1.5 to 2, and of diminishing doses ensuing in 3 to 4 minutes, the haemodynamic responses were registered, starting with the registration of the response of normal coronary flow to beta-adrenergic stimulation under normal conditions. After a pause of 35 to 40 minutes the anterior descending coronary branch was occluded and after stabilization of collateral blood flow level the dose-response curves were determined again. The number of successive infusion periods as well as the upper dose-levels were limited by the stability of the preparation. However, for the construction of the individual curves the response was studied at least in three different ranges. Doses in the range of 0.2 $\mu gkg^{-1}min^{-1}$ and 0.8 $\mu gkg^{-1}min^{-1}$ were applied in all experiments under both normal and ischaemic conditions and statistical analysis was based on the responses to these doses. The effect of the maximum dose (1.6 $\mu g/kg^{-1}min^{-1}$) was studied in 8 of the 10 animals.

The results were evaluated statistically by Student's *t*-test.

Results

The results clearly indicated that the response of the collateral system to a beta-adrenergic stimulus basically corresponds to that of the normal coronary system. The dose-response relationships of the calculated vascular conductance

reflecting dilatation of the coronary system are represented in Fig. 1, indicating the dose-response curves from every individual experiment. Beta-adrenergic reactivity of local myocardial as well as of coronary sinus flow, is shown. In the majority of the experiments, coronary occlusion was followed by a marked decrease in local myocardial flow. However, in a few instances, especially when

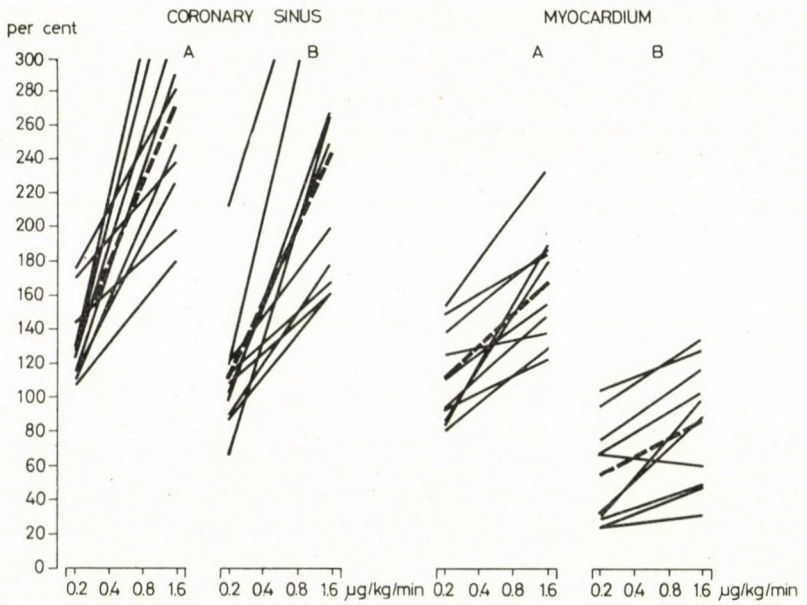

Fig. 1. Response of coronary vascular conductance to isoprenaline. Dose-response curves, left side, on the basis of coronary sinus flow; right side, on the basis of myocardial flow. A: normal state, B: myocardial ischaemia, - - - - - - - averaged dose-response curves, ——— individual dose response curves

the thermal probe had been fixed in the apical region, the resting level of the collateral blood supply hardly differed from that of the normal flow. In all but one experiment, the administration of isoprenaline was followed by a marked dilatation of the collaterals. This dilatation was unrelated to the resting flow level. At the same time, the dilator collateral response was slightly weaker than the local reaction of normal coronary sections supplying the same ventricular area. The average slope of the dose-response curves in the ischaemic state differed significantly ($p < 0.05$) from that of the controls.

Overall vasodilator responses of the coronary system were found to be more pronounced than those of the tissue blood flow, a phenomenon described by us earlier (JUHÁSZ-NAGY and SZENTIVÁNYI 1973). On the other hand, after the production of ischaemia, only slight modifications were demonstrable in the overall flow response, although the doses-response curves were generally

shifted to the right in consequence of some lowering of the baseline of blood flow. The decline in the average slope of the curves was, however, not significant statistically ($p > 0.50$).

A typical experiment including the dose-response curves is shown in Fig. 2 and the statistical evaluation of the responses in Tables I and II. It is seen that, with the exception of the modifications of flow in the directly affected ventricular segment, coronary occlusion in itself failed to affect significantly the resting values of the examined haemodynamic parameters, a finding reflecting, apart from the remarkable stability of the preparation, a considerable compensatory capacity of the coronary circulation. Moreover, the reduction of total coronary flow was not commensurate with the extent of the ischaemic area. pO_2 tension and pH in coronary sinus blood and their responses to isoprenaline showed no characteristic modification after coronary occlusion. Nor had the ischaemia any definite hypotensive effect. On the other hand, in the period of ischaemia the hypotensive response to major doses of isoprenaline was enhanced. The increase in pulse pressure, a characteristic sign of a normal response to beta-adrenergic stimulation, was considerably reduced after the induction of myocardial ischaemia, in all likelihood because of an impaired responsiveness to inotropic stimuli of the affected myocardial area. In contrast, the chronotropic action of isoproterenol — at least as far as the higher dose levels are concerned — practically attained the value found under normal conditions.

Discussion

It has been proved by recent experimental evidence obtained in several animal species that, although local myocardial flow declines significantly after the occlusion of a major coronary branch, it decreases far less than it has been assumed in the light of the earlier doctrine of the "coronary end-arteries" (cf. SCHAPER 1971).

In fact, blood supply to the ischaemic area is provided by primary collateral communications opening up immediately after coronary occlusion. The present results are consistent with the above findings, as the mean values for collateral blood flow were even in excess of those generally found in the dog heart (SCHAPER 1971). However, views are still divided concerning this issue. It is certain that the value for myocardial collateral flow depends on the anatomical site of measurement (LINDER 1966; TÖRÖK et al. 1969), on the size of the obstructed coronary branch (URBANICS and JUHÁSZ-NAGY 1971), on the nature of anaesthesia (CHIMOSKEY et al. 1967) and, last but not least, on the method of measurement (SCHAPER 1971). In areas of adequate collateral supply, particularly in the apical region of the dog heart, flow values attaining

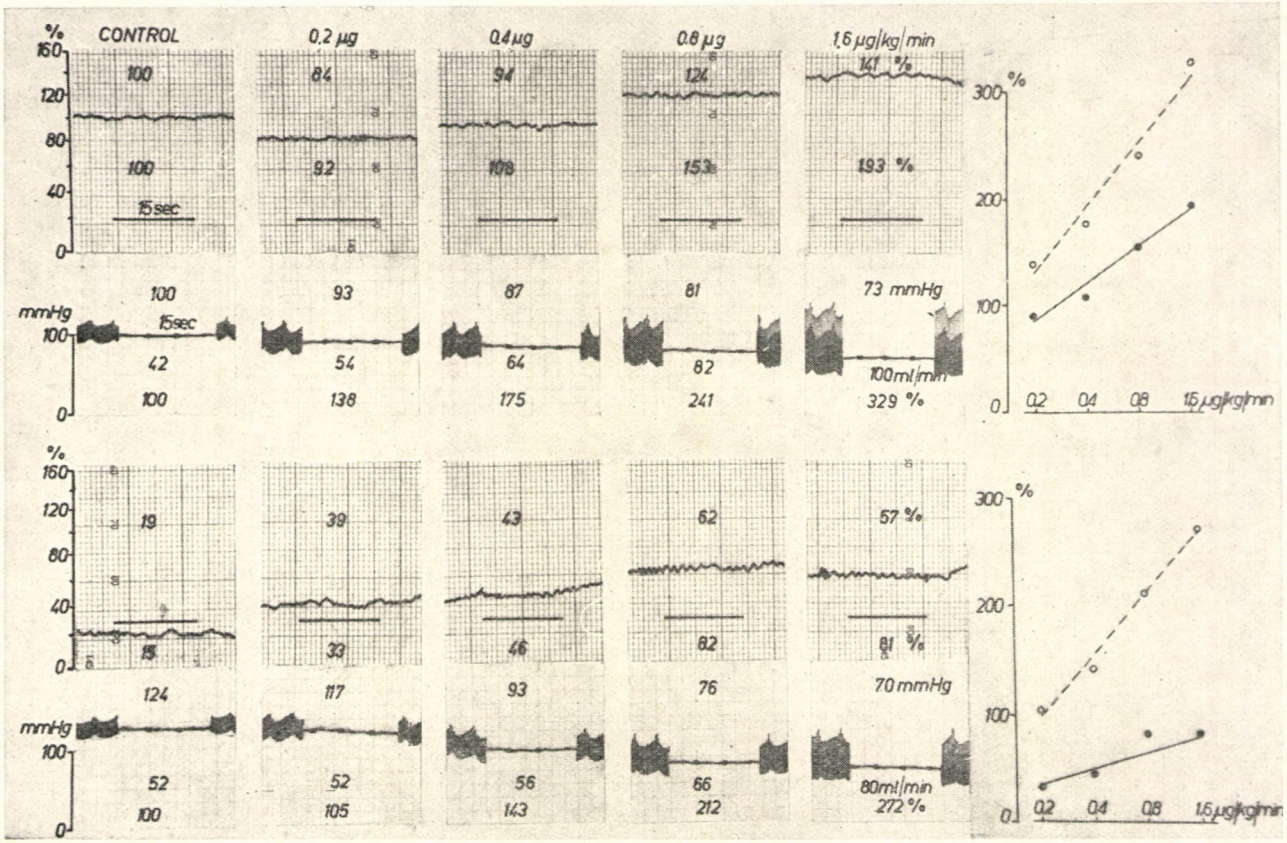

Fig. 2. Dog of 18 kg body-weight. Effect of isoprenaline infusion under normal conditions (upper part) and during myocardial ischaemia (lower part). Recording of myocardial blood flow at the top, of arterial blood pressure at the bottom of the individual blocks. Right margin: dose-response relationships of coronary vascular conductance. ○- - - - - - ○ coronary sinus flow, ○——○ myocardial blood flow. Numbering downwards: isoprenaline dose; myocardial flow (per cent); local vascular conductance (per cent) calculated on the basis of myocardial flow; mean systemic blood pressure (mmHg); coronary sinus flow (ml/min); vascular conductance (per cent) calculated on basis of coronary sinus flow The integrated middle sections of the blood pressure curves also mark the periods of coronary sinus flow measurement. The synchronous periods of myocardial flow registration are marked by horizontal lines

Table I

Haemodynamic responses ($n = 10$) to infusions of isoprenaline

	Normal state		
	control	0.2 μg kg^{-1}	0.8 μg kg^{-1}
Local myocardial flow, per cent	100	+ 8.5	+ 31.1
	± 0	± 6.0	± 13.1
Local conductance, per cent	100	+14.5	+ 49.2**
	± 0	± 7.5	± 11.7
Coronary sinus flow, ml min^{-1}	46.8	+14.9***	+ 41.7***
	± 3.3	± 1.9	± 5.2
Coronary sinus flow, per cent	100	+31.7***	+ 87.0***
	± 0	± 3.9	± 7.5
Sinus conductance, per cent	100	+38.7***	+120.1***
	± 0	± 6.5	± 14.0
Mean systemic blood pressure, mm Hg	112.3	— 4.1	— 14.5**
	± 8.7	± 1.9	± 4.4
Pulse pressure, mm Hg	27.1	+ 8.1***	+ 26.9***
	± 3.6	± 1.0	± 4.6
Heart rate, min^{-1}	171.0	+33.9***	+ 58.4***
	±12.1	± 4.7	± 7.4

Table II

Response of blood pO_2 and pH to infusion of isoprenaline

	Normal state		
	control	0.2 μg kg^{-1}	0.8 μg kg^{-1}
pO_2	22.0	+0.2	—1.2
Coronary sinus, mm Hg	+2.3	±0.9	±1.3
pO_2	87.6	—	—0.7
Arterial, mm Hg	±1.1		±1.2
pH	7.348	—0.014	—0.038**
Coronary sinus	±0.031	±0.005	±0.007
pH	7.394		—0.001
Arterial	±0.021	—	±0.003

(0.2 μg and 0.8 μg kg^{-1}min^{-1}). Mean values and ±S.E.M

	Myocardial ischaemia		
	control	0.2 μg kg^{-1}	0.8 μg kg^{-1}
Local myocardial flow, per cent	47.1	+ 4.6	+ 10.3
	± 8.6	± 2.6	± 6.7
Local conductance, per cent	49.6	+ 6.0*	+ 24.7**
	±10.4	-± 2.4	± 7.0
Coronary sinus flow, ml min^{-1}	43.3	+ 7.5**	+ 25.3***
	± 4.1	± 2.0	± 3.4
Coronary sinus flow, per cent	93.0	+17.0**	+ 52.8***
	± 6.4	± 5.1	± 5.2
Sinus conductance, per cent	93.3	+22.2**	+103.3***
	± 6.3	± 6.6	± 19.7
Mean systemic blood pressure, mmHg	110.1	— 3.5	— 21.7**
	± 6.7	± 2.2	± 5.9
Pulse pressure, mmHg	28.0	+ 3.4*	+ 13.4*
	± 2.0	± 1.6	± 5.3
Heart rate, min^{-1}	175.6	+18.5***	+ 48.6***
	± 9.6	± 3.9	± 9.9

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

(0.2 and 0.8 μg kg^{-1}min^{-1}) (n = 5). Mean values and ±S.E.M.

	Myocardial ischaemia		
	control	0.2 μg kg^{-1}	0.8 μg kg^{-1}
pO_2	24.1	—1.2	—1.4
Coronary sinus, mmHg	±4.1	±2.7	±5.1
pO_2	89.7	—	—0.9
Arterial, mm Hg	±2.3		±1.6
pH	7.294	—0.012	—0.038*
Coronary sinus	±0.028	±0.007	±0.011
pH	7.378		—0.001
Arterial	±0.026	—	±0.004

* $p < 0.05$ ** $p < 0.01$

or even exceeding normal levels are common (COULSON et al. 1970). It has been demonstrated by recent studies under strictly controlled experimental conditions that after coronary occlusion the affected region of the dog heart not only preserves some of its contractile force (its muscular tension thus being not merely a passive product of the contractility in the surrounding unaffected myocardium) but also that this residual muscular capacity is still capable of being further increased by adrenergic impulses (SCHELBERT et al. 1971). The regulatory mechanisms of myocardial circulation in the ischaemic zones are therefore of obvious importance.

Yet, although there are some amply confirmed data concerning the regulation of flow in the normal coronary system, the regulatory mechanisms of coronary collateral flow are poorly understood. According to the traditional view (KATTUS and GREGG 1959), the collaterals represent passive vascular channels. This view was sharply contradicted by present findings, since the beta-mimetic effect, the most important neurohumoral mechanism responsible for the regulation of coronary flow, involved also the collateral circulation. Furthermore, the collateral vasodilation was unrelated to the resting level of flow which varied within a broad range according to the site of measurement. The beta-adrenergic dilatation of the collaterals supplying the ischaemic area amounted to about 50% of normal coronary dilatation.

Considering the facts that the A—VO_2 difference prevailing in the coronary bed under resting conditions is not below 70%, and myocardial hypoxia is the most potent stimulus of dilatation in this vascular field, it is safe to assume that the microvasculature of the ischaemic area, at least in the case of resting values of less than the normal 70 to 75%, was in a state of maximum dilatation from the very outset. Under these conditions a further increase in the blood supply of the ischaemic area can only be achieved by a dilatation of those arterial communications which are situated in the marginal zone, in the first place in the unaffected circulatory areas. It may be assumed that since these collaterals had not been affected by the local hypoxia, their adaptational capacity was not exhausted and so they were still capable of further dilatation in response to beta-mimetic impulses, in the same manner as were the coronary sections distant from the ischaemic area.

In the course of the present study local ischaemia, though involving a considerable part of the left ventricle, produced no noteworthy change in the resting A—VO_2 difference, nor did it affect the cardiac O_2 extraction under the influence of beta-adrenergic stimulation. Although the isoprenaline-induced increases in vascular conductance reflecting changes in the overall coronary circulation remained slightly below the values characterizing the normal vascular response, the slopes of the dose-response curves were largely similar in the ischaemic and the normal state. These facts, though indirectly, also point to the remarkable adaptability of the vascular segments responsible for the

maintenance of collateral circulation, since the vasodilation ensuing in response to beta-adrenergic stimulation had to be "superposed" on an adaptational vasodilation directed at the correction of the ischaemic state. It is obvious, moreover, that this adaptation could not be imposed uniformly on the entire coronary system but primarily on the vasculature adjacent to the ischaemic area, even though a general functional hyperaemia may have also had a part in its production, since the function of the ischaemic ventricular segment is taken over by the unaffected ventricular musculature as a whole. At any rate, however, even under resting conditions, there had to be an adaptational gradient prevailing between the intact ventricular areas distant from the ischaemic area and those adjacent to it (i.e. furnishing the collaterals) and no significant decrease in this gradient could have been produced by the gradual increase in beta-adrenergic tone. Otherwise, that is in the absence of an internal coronary coordination of this kind, the beta-adrenergic stimulation would have had to be followed, instead of a vasodilatation by the reverse of it, namely by a steal phenomenon within the ischaemic area. This phenomenon was, however, confined to a single preparation in our study and even there it was not significant (Fig. 1).

An internal coronary steal phenomenon has been described recently in experimental myocardial ischaemia by SHARMA et al. (1971) and by UCHIDA and UEDA (1972). The conditions of its production are, however, still controversial. In the light of the present study, active coronary dilatation of the unaffected ventricular areas offers no satisfactory explanation for the coronary steal. Production of this phenomenon presupposes some disorder of the coronary adaptation additional to a simple hydrodynamic mechanism.

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ABSORPTION AND TRANSPORT OF PROTEIN FROM THE PERITONEAL CAVITY

By

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Absorption of ^{131}I-albumin from the diaphragmatic aspect of the peritoneum, from the liver and the intestinal serosa was measured in dogs. Protein absorption from the liver surface was about four times more rapid than that from the diaphragm. No protein was absorbed from the gut serosa. About 40% of the absorbed protein entered directly into the blood capillaries. The lymphatics joining the great veins at the right side of the neck carried about twice as much absorbed protein as did the thoracic duct.

About 20 years ago, when COURTICE and SIMMONDS [3] reviewed the physiology of serous membranes, it seemed firmly established that protein molecules are absorbed from the abdominal cavity mainly on the peritoneal aspect of the diaphragm and are then transported into the circulation almost exclusively by the lymph vessels. At closer scrutiny it seems, however, that this assumption has been based on slender evidence.

The site of absorption from the peritoneal cavity was investigated mainly with particles, such as graphite, colloidal silver, red cells, etc. [1, 8, 9, 12]. There are only few reports on an intense staining of the diaphragm after the intraperitoneal injection of dye-labelled plasma protein [5, 6]. From these observations it has been concluded that the diaphragm was the site of protein absorption.

On the other hand, various particles are carried away from the serous cavities by the lymph vessels. From this observation again no conclusions should be drawn as to the transport of protein molecules. There is no doubt that the small molecules are exchanged across the endothelial and mesothelial membranes by a diffusion process and evidence is now available that in the blood capillaries, there may occur a similar exchange of colloid molecules [13—16]. The assumption that no substantial amounts of protein are absorbed from the peritoneal cavity into the blood capillaries is based on a single observation made in 3 rats with the injection of T-1824-labelled serum [5].

From the clinical point of view, in the understanding of the formation and resolution of peritoneal effusions, protein absorption and transport from the peritoneal cavity is of considerable interest. Accordingly, a study of this problem seemed to be warranted.

Material and Methods

Investigations were made on 58 mongrel dogs under pentobarbital anaesthesia (initial dose, 30 mg/kg). The abdomen was opened by a midline incision. Then ^{131}I-labelled human albumin (total radioactivity about 5 μCi), dissolved in 0.1 ml 2% albumin solution was introduced into the peritoneal cavity. The material was dialysed and checked to contain less than 2% of uncoupled free ^{131}I. The protein solution was applied on a round piece or strip of filter paper (surface 3 to 4 sq. cm.).

1. Absorption from the peritoneal aspect of the diaphragm

A round filter paper 2 cm in diameter with ^{131}I-albumin was placed on the diaphragm anteriorly and to the left of the vertebral column. To prevent contact with the adjacent structures the filter paper was covered with a sheat of plastic. Then the abdomen was closed and lymph and serum samples were collected. At the end of the experiment the filter paper and a large portion of the diaphragm were removed and measured for radioactivity.

Group A. In 15 dogs the thoracic duct and the right lymph trunk were directly cannulated in the neck with a teflon tube. The difficulties of right duct cannulation are well known. An indirect approach proved to be entirely unsatisfactory. Therefore, cannulation of a main branch of the right duct was attempted in every animal. If such a vessel was not found or if its cannulation was unsuccessful the animal was discarded or the procedure described below (Group B) was adopted. Lymph was collected every hour for 6 hours and at the end of each collection period an arterial blood sample was taken.

Group B. In another group of 8 animals the thoracic duct was cannulated in the left side of the neck. On the right side the subclavian and the innominate veins were bared and isolated. The surrounding tissues were sectioned between bulk ligatures. By this procedure all lymphatic and venous channels communicating with the great cervical veins were severed.

In these animals thoracic duct lymph and arterial blood was collected as in the previous group.

2. Absorption from the liver surface

A round piece of filter paper with labelled albumin solution was placed on the anterior surface of the left lobe of the liver and covered with plastic.

Group A. In 11 dogs lymph from the thoracic and the right duct was collected as in Group 1A.

Group B. In 9 dogs the same procedures were applied as in Group 1B.

At the end of the experiment the filter paper and the adjacent parts of the liver were removed for radioactivity measurement.

3. Absorption from the peritoneum covering the jejunum

In 15 dogs the labelled protein was applied on a 0.5×6 cm strip of filter paper placed around the jejunum at a distance of 30 to 40 cm from the duodenum. The paper was isolated from its surroundings by a sheat of plastic. At the end of the experiment the filter paper and adjacent parts of the intestinal wall were removed for radioactivity measurement.

In 8 of these animals again the thoracic duct and the right trunk were cannulated. As in these animals the right trunk did not carry appreciable amounts of radioactive protein, in the remaining 7 dogs only the thoracic duct was cannulated and no attempt was made to interrupt lymph inflow into the veins at the right side of the neck.

At the end of the experiment, circulating plasma volume was measured with the Evans blue dye-dilution method. Tissue samples and the filter paper removed from the animals were treated with 20% sodium hydroxide. Radioactivity in lymph and plasma samples as well as in aliquots of the digested tissues and the filter paper was measured in a well type scintillation detector. The amount of ^{131}I-albumin present in circulating blood plasma was calculated from the proteinbound radioactivity and plasma volume. ^{131}I-albumin transported by the lymphatics was calculated from the radioactivity found in the collected lymph samples. The results were expressed as per cent of the total introduced radioactive dose. The values given in the text and figures are means $\pm$ standard error.

Results

1. *Absorption from the diaphragm* was slow (Fig. 1). In 6 hours, only $1.6 \pm 0.4\%$ of the introduced radioalbumin was recovered in the thoracic duct lymph; $1.9 \pm 1.2\%$ was found in the lymph flowing from the cannulated right duct and $8.6 \pm 2.2\%$ was recovered from the circulating plasma, giving a total of 12.1% for diaphragmatic absorption. $58.7 \pm 12.6\%$ of the material remained on the filter paper or in the adjacent tissues.

In the animals with ligated right lymph trunk the fraction transported in 6 hours by the thoracic duct was $2.4 \pm 0.9\%$ and 4.7 ± 0.8 was recovered in the circulating plasma, giving 7.1% as a total for the 6 hr absorption (Fig. 2).

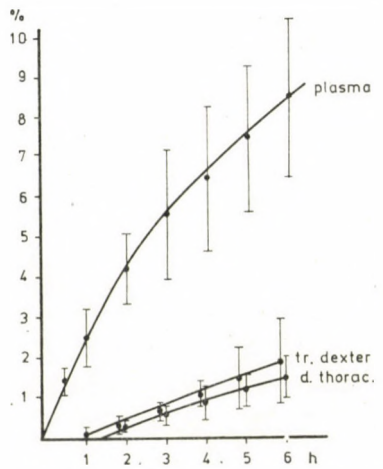

Fig. 1. Absorption of ^{131}I-albumin from peritoneal aspect of the diaphragm (Per cents of the introduced dose recovered from circulating blood serum, thoracic duct and right lymph trunk lymph)

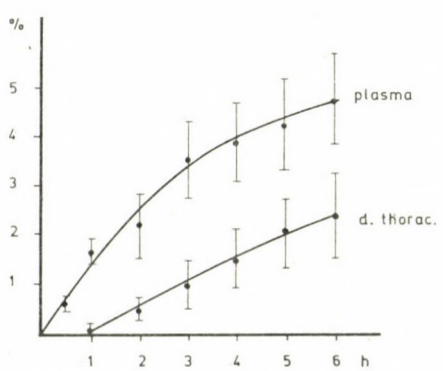

Fig. 2. Absorption of ^{131}I-albumin from diaphragmatic aspect of peritoneum in dogs with ligated right lymph trunk (Per cents of dose found in serum and thoracic duct lymph)

The amount which remained unabsorbed at the site of application was 58.0 ± 8.7%.

A comparison of the two groups made it clear that the lymphatics terminating on the right side of the neck are probably the most important pathway for the transport of the protein absorbed at the peritoneal aspect of the diaphragm. This is demonstrated in Fig. 3, where the amount of the

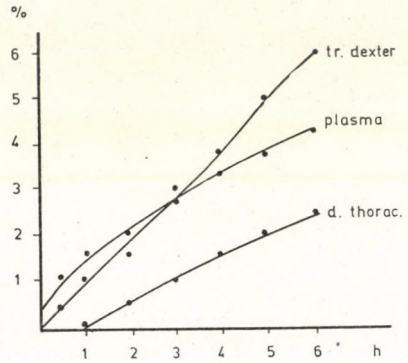

Fig. 3. Absorption of ^{131}I-albumin from diaphragm. Calculated transport by lymph vessels on the right side of the neck ("tr. dexter")
(Further explanation in text)

labelled protein entering the veins at the right side of the neck ("tr. dexter") is calculated by adding to the differences of the plasma ^{131}I-albumin values of groups A and B, the amounts recovered in the right lymph duct lymph of group A. In the graph the values for thoracic duct lymph and blood plasma are those of group B.

2. *Absorption from the liver surface* was found to be more rapid than the absorption from the diaphragm. After 6 hr, the total amount found in plasma and lymph was 50.5% and only 3.1 ± 1.0% was recovered at the site of application. The fraction carried away by the thoracic duct lymph was 7.1 ± 1.3%, while 9.3 ± 1.9% was found in the right lymph trunk and 34.1 ± 5.0% in the circulating plasma (Fig. 4).

In the animals with ligated right duct (Group B), recovery in circulating plasma dropped to 18.7 ± 2.5, and in 6 hr 9.3 ± 2.6% was transported by the thoracic duct (Fig. 5). Accordingly, the total amount found in plasma and lymph was in these animals 28% of the introduced dose. The importance of the lymphatics on the right side of the neck in the transport of protein was estimated in the same manner as in the previous experiments. Fig. 6 shows that again these channels are probably the most important pathway of protein absorption.

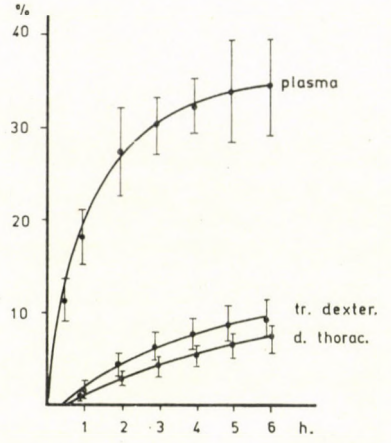

Fig. 4. Absorption of ^{131}I-albumin from liver surface
(Per cents of the dose found in serum, thoracic duct and right lymph trunk lymph)

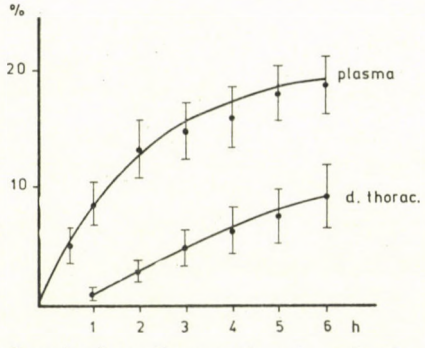

Fig. 5. Absorption of ^{131}I-albumin from liver surface in animals with ligated right lymph trunk
(Per cents of introduced dose found in serum and thoracic duct lymph)

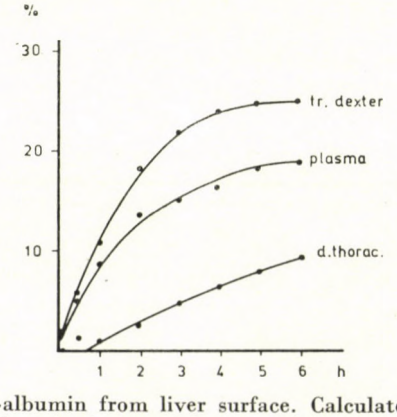

Fig. 6. Absorption of ^{131}I-albumin from liver surface. Calculated amount of transport by lymphatics on right side of the neck
(Further informations in text)

3. *From the serosa of the jejunum* there was practically no absorption. After 6 hr only 2.9% of the applied amount was found in the circulating plasma and the thoracic duct lymph. In the right lymph trunk ^{131}I-albumin concentration remained below its plasma concentration, consequently no absorbed protein is transported by this route (Fig. 7).

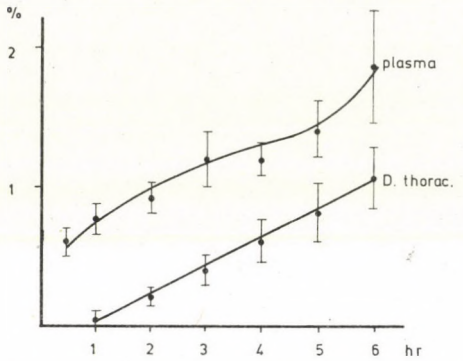

Fig. 7. Absorption of ^{131}I-albumin from intestinal serosa

In view of the small amounts of label found in plasma and lymph, there is no evidence that this protein is absorbed from the peritoneal surface of the small intestine. Actually, the absorption may have occurred from some other part of the peritoneum after the partial elution of the protein from the filter paper by the peritoneal fluid. This assumption is corroborated by the fact that at the end of the experiment only 20.4 $\pm$ 3.7% of the label was found on the filter paper and in the adjacent portion of the gut wall.

Discussion

The experiments reported showed that the peritoneal aspect of the diaphragm is not the only and not even the most important site of protein absorption from the abdominal cavity. Plasma protein is absorbed with considerable speed also from the capsule of the liver. Whether the rate of absorption per unit area from the liver surface really exceeds about four times that from the peritoneal aspect of the diaphragm, is not easy to decide. It has been reported [2, 6, 9] that there are substantial regional differences in the activity of the diaphragmatic absorbing surface. In the present experiments, the application site of the labelled protein was chosen for its convenience and accessibility. It is quite possible that in the dog this is a relatively inactive area of the diaphragm. Another source of error could be that the contact between

the moving diaphragm and the introduced protein is less intimate and constant than on the immobile liver surface. These considerations cannot, however, influence the fact that the liver surface is a site of rapid protein absorption.

As to the importance of these findings in physiology and pathology, there is an oversimplification which should be dealt with. The normal process of peritoneal fluid formation or the accumulation of effusions is usually described in terms of escape from the blood capillaries of a fluid with a determined composition and of its absorption into the lymph vessels in some specific area of the peritoneum. This is, however, only the final consequence of a complicated process. Net fluid and solute flow is a result of the diffusion of crystalloid and colloid molecules across the vessel walls and the peritoneal membrane, the filtration of the solvent from the capillaries, uptake of the fluid by the terminal lymphatic vessels, etc. These processes are governed by different and independent hydrostatic and osmotic forces, and they may occur on different parts of the peritoneum. The composition of normal peritoneal fluid reflects an equilibrium between capillary filtration and absorption, exchange by diffusion of the individual molecules and bulk absorption by the lymphatics. The liver capsule seems to be, beside the diaphragmatic aspect of the peritoneum, the most important site of this exchange, at least in respect of the protein molecules. Pathologic fluid accumulations are the result of a perturbed equilibrium. In their most common variety, in ascites produced in Laennec's cirrhosis by postsinusoidal venous stasis, the liver capsule is actually the place of fluid and protein leakage [10, 11]. It was suggested that this fluid of high protein content is filtered not, or not only, from the liver sinusoids but also from the engorged capsular lymphatics [10, 17]. In this condition, the escaped fluid is reabsorbed obviously not from the liver surface but from some other part of the peritoneum.

In the present experiments protein absorption from the serosa covering the small intestines was also measured. This site was selected on the basis of the assumption, that in some cirrhotic patients fluid and protein escaping from the intestinal wall could be the cause of ascites formation [7]. Our results have, however, made to seem rather improbable that protein diffusion — and for that matter also filtration — would occur across the intestinal capillaries and the serosa of the gut wall.

From our experiments it could also be concluded that protein may be removed from the peritoneal space by absorption into blood capillaries. It was found that at the peritoneal aspect of the diaphragm the ratio of venous versus lymphatic transport (calculated from both series of experiments) was 0.66 and the same ratio for absorption from the liver capsula was 0.59. This means that nearly 40% of the total absorbed protein entered directly the blood capillaries.

The lymphatics from the diaphragm and the liver capsule are joining predominantly the collecting paths on the right side of the neck, and not the thoracic duct. Actually, both from the diaphragm and from the liver surface about twice as much absorbed protein is transported by the tributaries of the right duct as by the thoracic duct.

The inferior part played by the thoracic duct in the transport of the absorbed protein and particles from the peritoneal cavity has been described previously in guinea pigs, cats and rabbits [4].

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SODIUM EXCRETION AFTER SALINE INFUSION UNDER NORMAL CONDITIONS AND IN THE SUCCESSIVE STAGES OF HYPERTENSIVE DISEASE

By

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The natriuretic response to hypertonic (5%) saline infusion has been studied in 112 subjects. After the acute salt load, a significant difference in natriuresis was noted between the hypertensive subjects and the normal controls. The difference was likewise significant between the normal controls and the normotensive subjects of the hyperreactor (NAT positive) group. No significant difference in reactive natriuresis was found between the hypertensive and the hyperreactor group.

Sodium excretion by hypertensive subjects after an acute salt load has widely been studied with the conclusion that such patients excrete more of the infused NaCl and at a higher rate than do normotensive individuals. We have investigated the problem in the different stages of hypertensive disease.

Material and Methods

The material consisted of 112 subjects; they were divided into the following groups.

I. Normal subjects without any evidence of heart or circulatory disease.

II. Hypertensive patients.

II/a. A particular group was formed by elderly patients with arteriosclerosis. These were divided into two subgroups, the first consisting of 6 patients with high-amplitude hypertension, diastolic pressures being in the range of 95 to 100 mmHg ("sclerotic hypertension, type I"); and the second of 4 patients with high-amplitude hypertension, diastolic pressure ranging from 70 to 90 mmHg ("sclerotic hypertension, type II").

III. Normotensive subjects with vegetative dystonia and a positive noradrenaline test, i.e. exhibiting a significant elevation (by more than 20 mmHg) of arterial blood pressure in response to the intravenous administration of a single dose of 5 μg noradrenaline. (For details, see Komor and Polgár, 1965.)

IV. Normotensive subjects with vegetative dystonia and a negative noradrenaline test, thus exhibiting no significant rise in blood pressure in response to noradrenaline.

Antihypertensive drugs were withdrawn and only mild sedatives allowed during the last two weeks before and during the salt loads. Patients with heart or kidney failure or cerebrovascular lesions were excluded from the studies. Daily dietary sodium intake was restricted to 3 to 5 g. During a preliminary test period of three days, 24 hr urines were collected for three successive days and urinary Na, K and Cl were measured in the pooled urine. In order to check the reliability of collection, endogenous creatinine clearance was also determined. The salt loading tests were performed on two successive days, 25 g NaCl being administered daily in the form of a 5% intravenous infusion at a rate of 60 to 80 drops per minute. Collection of urine was continued during and after the loading for another three days.

K and Na concentrations were measured with a Zeiss III flame photometer, using concentrated ammonia in 100-fold dilution as diluent. For the estimations of K 100-fold,

for those of Na, 50-fold urinary dilutions were used. Cl-ions were measured according to SCHALES by means of mercurometry with diphenylcarbazone as indicator. Urinary creatinine was estimated by the JAFFE reaction: in alkaline medium, in the presence of saturated picric acid, creatinine is converted to sodium picrate reddish-brown in colour, the intensity of which is related to the amount of creatinine. In general, 100-fold urinary dilutions were used.

Results

Group I. Controls, i.e. normotensive individuals, with no evidence of cardial, renal or hepatic disease and with a negative family history for hypertension (30 subjects).

Daily sodium excretion amounted from 3.21 to 3.67 g prior to the tests and attained 7.56 to 8.49 g in response to NaCl-loading. In the course of three

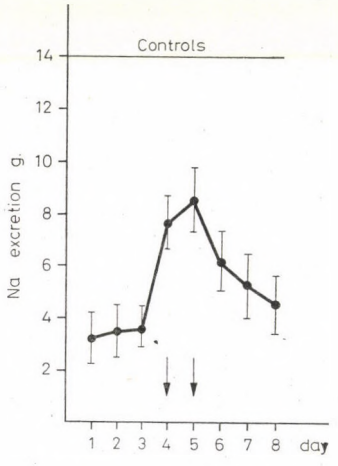

Fig. 1. NaCl excretion in 30 normal controls, in response to two infusions of 25 g $NaCl$ (= 19.6 g Na) each on 4th and 5th day

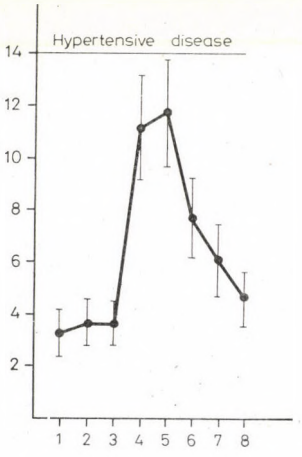

Fig. 2. NaCl excretion in 51 hypertensive subjects in response to NaCl-loading (see legend of Fig. 1)

days after the tests it successively diminished to 6.15, finally to 4.55 g (Fig. 1). Blood pressure increased during the period of study including the preliminary stage, the loading tests and the subsequent three days, to 7.3 systolic and 11.12 mmHg diastolic value. As column 1 of Fig. 7 shows, blood pressure remained slightly below the original level for a few days after completion of the loading tests (Fig. 7).

Group II. The hypertensive group comprizing patients included 10 elderly patients in stages III—IV of sclerotic type hypertension with a response different from that of the other 51 hypertensive subjects (see Group II/a). Natriuresis of the 51 subjects in the course of the 3 days prior to loading ranged from 3.27 to 3.62 g, and from 11.13 to 11.72 g in response to NaCl-loading. It declined thereafter to a mean value of 7.63, then to 4.61 g in the

course of the first three days after loading (Fig. 2). The hypertensive response was systolic as well as diastolic in all but 2 cases of this group, the mean rise in systolic pressure being 12.7 and in diastolic pressure, 24.0 mmHg (see column II in Fig. 7). It was likewise in this group that occasional adverse

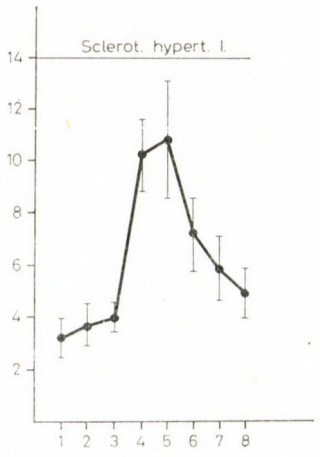

Fig. 3. NaCl excretion in response to NaCl loading in 6 subjects with arteriosclerotic hyper tension (diastolic pressure from 95 to 100 mmHg)

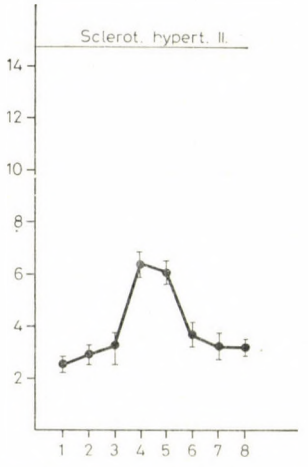

Fig. 4. NaCl excretion in response to NaCl loading in 4 subjects with arteriosclerotic hypertension (diastolic pressure below 90 mmHg)

reactions were noted, although the hypertonic saline infusion was tolerated well by most patients.

Group II/a. In this group of sclerotic hypertension the response to NaCl-loading was inconsistent. In 6 of the 10 subjects the response was similar to that found in the hypertensive group, with the difference that total sodium excretion on the second day of loading was less than in the former group (Fig.3). In the other 4 cases, the loading failed to elicit a typical response. These cases may thus be regarded as unresponsive. In fact, sodium excretion on the second day of loading failed to attain the figures found in the controls (Table I).

Group III. This group was formed by 14 noradrenaline-positive subjects with vegetative dystonia. The vegetative symptoms were in many respects similar to those of hypertension. All patients initially were normotensive. In the noradrenaline test, 14 out of 21 gave a positive reaction.

The response of the 14 reactors was similar to the reaction of the hypertensive patients (Fig. 5). In the three days before loading, mean sodium excretion ranged between 2.96 and 3.63 g, attaining 12.78 to 13.23 g in response to NaCl-loading and declining successively to 8.97 and 4.36 g, in the course of

Table I

Salt excretion (mean ± SD) as a function of time (days). Loading on 4th and 5th days

Group	Day	1	2	3	4	5	6	7	8	4/3 day ratio	*N*
I.	Controls	3.21	3.49	3.67	7.56	8.49	6.15	5.21	4.55	2.17	30
		±1.01	±1.02	±0.79	±0.97	±1.30	±1.15	±1.21	±1.11	±0.59	
II.	Hypertension	3.27	3.64	3.62	11.13	11.72	7.63	6.05	4.61	3.19	51
		±0.89	±0.95	±0.85	±2.09	±2.08	±1.55	±1.39	±1.08	±0.72	
II.a.	Sclerotic hypertension I	3.18	3.67	3.95	10.25	10.83	7.20	5.88	4.97	2.64	6
		±0.69	±0.83	±0.49	±1.42	±2.36	±1.49	±1.22	±0.93	±0.57	
II.a.	Sclerotic hypertension II	2.55	2.93	3.23	6.33	6.03	3.70	3.23	3.15	2.00	4
		±0.24	±0.26	±0.57	±0.38	±0.39	±0.42	±0.50	±0.26	±0.28	
III.	Noradrenaline positive	2.96	3.70	3.63	12.78	13.23	8.97	5.63	4.36	3.56	14
		±0.45	±0.62	±0.42	±1.23	±1.12	±1.51	±0.87	±0.83	±0.53	
IV.	Noradrenaline negative	3.79	3.91	3.93	9.59	8.24	6.57	5.30	3.87	2.46	7
		±0.58	±0.30	±0.45	±0.46	±0.58	±1.01	±0.60	±0.71	±0.27	

the three days subsequent to loading. The average hypertensive response to NaCl-loading was 21.1 mmHg systolic and 36.1 diastolic in the 14 subjects of this group (see Fig. 7, Group III). Headaches, dryness of mouth were reported after the loading tests by the majority of patients; 8 patients complained of weekness in the limbs, 6 of mild oppression.

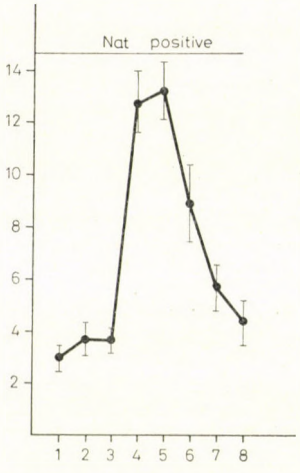

Fig. 5. NaCl excretion in response to NaCl loading in 14 normotensive noradrenaline-positive subjects (reactors)

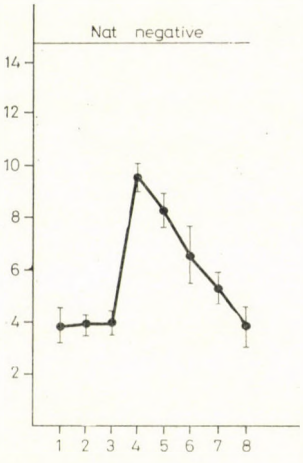

Fig. 6. NaCl excretion in response to NaCl-loading in 7 normotensive, noradrenaline-negative subjects with vegetative dystonia

Group IV. In the 7 noradrenaline-negative cases, the reaction to the loading was similar as in the normal controls (Table I, Fig. 6). The hypertensive response too, was basically the same as in the controls (see Fig. 7, Column IV).

Discussion

It is some 70 years ago that French authors have first pointed to the hypotensive effect of a low salt diet: restriction of dietary sodium intake or administration of saluretic agents is usually followed by a fall in blood pressure. There is extensive experimental evidence suggestive of the existence of connections between hypertensive disease and sodium. Arterial hypertension can be induced by massive amounts of sodium administered together with desoxycorticosterone and SARRE (1944) showed this effect to be associated with an increase in peripheral resistance. These findings stimulated further studies; interpretation of the results was, however, difficult as HOLLAENDER and JUDSON (1957) obtained similar results with isotonic, some other authors with hypertonic saline solutions, again others, e.g. BRODSKY and GRAUBARTH (1953), with mannitol infusion.

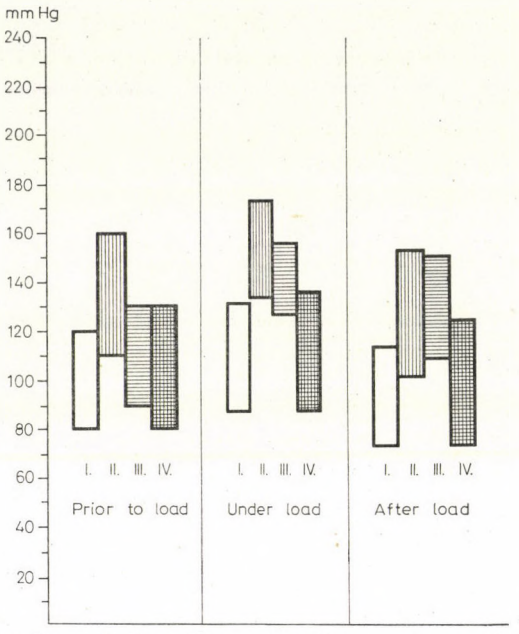

Fig. 7. Systolic and diastolic pressure in response to NaCl loading
I: mean of 30 normal controls
II: 51 hypertensive subjects
III: 14 noradrenaline-positive normotensive subjects with vegetative dystonia
IV: 7 noradrenaline-negative normotensive subjects with vegetative dystonia

GREEN et al. (1952) induced an increase in diuresis and natriuresis in 6 subjects by infusing hypertonic (5%) saline; blood pressure increased parallel with natriuresis. THOMPSON et al. (1954) infused 2.5% saline to hypertensive subjects; NaCl was excreted in larger amounts and at a higher rate than in normal controls. BALDWIN et al. (1958) noted an excessive natriuresis not only after infusions of 5% saline but also after those of glucose, inulin and PAH in 20 subjects with essential hypertension; tubular sodium reabsorption remained normal in these patients.

COTTIER et al. (1958), examining the response to 2.5% saline of hypertensive and normotensive individuals, found the maximum increase in natriuresis in the hypertensive patients in the first 3 hours of loading.

Subsequently, COTTIER (1960), DAHL et al. (1960, 1961), MERTZ and SARRE (1964), and others have shown that hypertensive disease is marked by a paradoxical response to hypertonic saline infusion, as more of the load is excreted and at a higher rate than in normal subjects.

These studies of fluid and salt balance were mostly confined to patients with manifest hypertension and to normal contros. COTTIER et al., examined the natriuretic responses in 8 patients with labile hypertension in the normo-

tensive period, and found a response similar to that given by most normotensive subjects. In contrast, AVIRAM et al. noted a hypertensive pattern of diuretic and saluretic response in 25 subjects with "potential hypertension", i.e. in 10 patients with earlier toxaemia of pregnancy and in 15 with labile hypertension.

In most of the clinical observations and animal experiments it was attempted to correlate the naturiuretic response with changes in renal haemodynamics. Various theories were advanced, but none was supported by adequate experimental evidence. Recent findings (MROCZEK et al. 1970) have connected the hypertensive type of natriuretic response to a salt load with a sympathetic reaction since it could be inhibited by betalytic agents such as propranolol.

It seems to be new in the present observations that normotensive hyperreactors to noradrenaline, i.e. those in whom the drug elicits a hypertensive reaction, exhibit a paradoxical sodium excretory pattern similar to that observed in hypertensive subjects. Our findings indicate that a disturbance of renal sodium and water excretion is present in the early phase of hypertensive disease already and asserts itself in an increasing degree with the progress of the process but tends to be normalized in the sclerotic phase. In our sclerotic patients over 60 years of age, sodium excretion declined parallel with the decrease in diastolic blood pressure and diminished gradually below the control values with the advance of the process (Figs 3, 4).

For statistical evaluation, the 4/3-day ratio, i.e. that of the values found prior to loading on the 3rd day and on the first day of the test (4th day) was calculated for each group. A significant difference was found between the controls and the hypertensive, as well as the noradrenaline-positive normotensive subjects, whereas the difference between the controls and the noradrenaline-negative normotensive individuals was not significant. Parallel with the advance of age (and of the process) the difference in natriuresis windles and the patients with low diastolic pressure show again no significant difference from the controls in respect of salt excretion (Table II).

Table II

Statistical evaluation of the 4/3-day ratio

Groups	Value	Significance level
Control-Hypertension	7.70	$< 0.1\%$ significant
Control-Sclerotic Hypertension I.	1.77	$> 5.0\%$ not significant
Control-noradrenaline positive	7.48	$< 0.1\%$ significant
Control-noradrenaline negative	1.25	$>30\%$ not significant
Noradrenaline negative positive	5.03	$< 0.1\%$ significant
Hypertension noradrenaline negative	2.87	$< 1.0\%$ significant
Hypertension noradrenaline positive	1.81	$> 5.0\%$ not significant

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GUINEA PIGS IMMUNIZED WITH XENOGENEIC CELLS: SKIN TEST, IN VITRO STIMULATION, MIGRATION, CYTOTOXICITY OF LYMPH NODE CELLS

By

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The lymphocyte response in three in vitro tests: blast transformation, inhibition of migration and cytotoxicity was compared with the cutaneous test in the early period of delayed type hypersensitivity. Guinea pigs were immunized with cells of human lymphoid line in Freund's adjuvant. Non-sensitized lymph node cells did not react with the xenogeneic cells in either test. High thymidine incorporation of the immune lymphocyte population and a relative faster migration without added antigen in vitro was recorded with maximum values on day 6. In the presence of the immunizing cells all the in vitro test were negative 2 days after immunization and maximum activity was found at day 6. These responses went parallel with the degree of positivity of the skin test. In spite of the fact that skin test and lymphocyte cytotoxicity could be evoked, the mixed lymphocyte target cell test was negative with mouse cells used as antigen (YCAB) or target cells (A9).

Introduction

In a number of in vitro systems designed to study delayed hypersensitivity, the performance of lymphoid cells correlate well with the animal's immune status. E.g. the extent of the skin reaction elicited by antigens went parallel with the induction of blast transformation of lymphocytes [3, 28, 32, 34], inhibition of migration of macrophage-lymphocyte mixtures [4, 5, 12, 16], and with the toxic effect of lymphocytes on the antigen carrying target cells [1, 6, 26, 33, 38, 40].

It seems thus that each function of the immunopotent cells studied by these in vitro techniques correlate well with skin reactivity and thus probably also with each other. Experiments performed with tuberculin sensitized guinea pigs pointed however to certain discrepancies while a good correlation between skin test and transformation of lymphocytes was found, the migration inhibition test did not correlate with them [5, 32]. Similarly, kinetic studies on skin test, migration inhibition and antibody production in the course of the development of delayed hypersensitivity also showed discrepancies [15, 20, 33].

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As these experiments involve several variable factors and relate to different antigens, the parameters of the immune response also seem to be variable [24]. The divergences are mainly seen in the early stage of delayed hypersensitivity, as in the late stage there is a good correlation between the in vitro test and the state of immune reactivity [18, 20, 34, 43].

These considerations have motivated us to study the stimulation of DNA synthesis, the migration inhibition, and the cytotoxic effect of lymph node cells in comparison to the skin test in the early stage of delayed hypersensitivity. As antigen, xenogeneic lymphoblastoid cell cultures were used.

Methods

Animals. Adult guinea pigs of both sexed, weighing 250 to 400 g, were used.

Cell lines. The human lymphoid cell line derived from Burkitt lymphoma (Daudi) was maintained in stationary suspension culture. The mouse line YCAB is a murine Moloney virus induced leukaemia cell line originating from $ACA \times AF_1$ mice was also carried in suspension. The MGB cell line was derived from a mouse methylcholantrene induced sarcoma in a CBA mouse. The A 9 mouse fibroblast line was derived from the L cell line by selection for 8-asaguanine resistance.

All cell lines were cultured and the tests were performed in MEM supplemented with 10% foetal calf serum.

Immunization. 0.5 ml 10^7 washed Daudi cells were mixed with equal quantities of complete Freund's adjuvant (Difco Laboratories, Michigan, USA) and injected into the rear footpads. In the control groups, the same amount of Freund's adjuvant was injected alone.

Skin test. The animals were injected intradermally in a shaved area on both sides of the abdomen with 0.1 ml containing 10^6, 10^5, 10^4, 10^3 washed cells in balanced salt solution (BSS). As a control, 0.1 ml of culture medium or complete Freund's adjuvant was used. The reaction was read 40 hr later and the diameter of erythema and infiltration was recorded.

Harvesting of lymph node cells. The guinea pigs were bled by heart puncture and killed by air embolisation. The inguinal and exillary lymph nodes from 3—5 guinea pigs were pooled 2, 4, 6, 8, 10, 12 days after sensitization. The whole protocol was repeated twice.

The lymph nodes were collected and the suspensions were prepared under sterile conditions. The nodes were cut in small pieces and suspended by gentle suction in 1 ml plastic syringe. After a few minutes, the sedimented cells in the supernatant were washed twice with MEM. Cell viability was assessed by trypan blue exclusion. Only cell suspensions which contained less than 15% of stained cells were used in the tests.

The lymph node suspension of individual animals was employed separately in the blast transformation study. For migration and cytotoxicity tests, the suspensions from different animals were pooled, divided and diluted according to the requirements of each test.

Induction of DNA synthesis. The stimulator cells Daudi, YCAB and CBA spleen cells were irradiated with 6,000 t (150 kW, 20 mA, 2 mm Al filter). The cells were washed before and after irradiation and suspended in culture medium.

The mixed cultures containing guinea pig lymph node cells and 10^5 Daudi, YCAB or MGB c lls, or 5×10^5 CBA spleen cells, were set up in a total volume of 2 ml. Lymph node lymphocytes were incubated alone or with PHA (The Wellcome Foundation Ltd., London) at a final concentration of 0.5% v/v. Tubes containing different irradiated stimulator cells alone or PHA or media were set up as controls. The tubes were incubated at 37 °C in humidified atmosphere containing 5% CO_2, for periods from 1.5 to 5.5 days. 24 hr before harvesting the culture 1 μCi of ^{3}H-thymidine (2Ci) mM (Tradiochemical Centre, Amersham) was added. The tubes were centrifuged at 2,000 r.p.m. for 5 minutes, the supernatant was discarded, the cell pellet was washed with 4.0 ml of DNA and precipitated twice with 4.0 ml of cold trichloroacetic acid. The precipitates were dissolved in 0.5 ml Hyamine (dimethylbenzylammonium hydroxide, Packard Instrument Co., Inc., Growe I 11) and the tubes were kept in darkness at room temperature for at least 5 hr. After addition of 0.8 ml methanol, the contents of the tubes were transferred to Packard counting vials containing 15 ml of scintillation fluid [15.2 g 2,5-diphenyloxazole (PPO), 0.380 g 4-bis-2-(5-phenyloxazolyl)-benzene

(POPOP), 30% Triton X-100, made up with toluol to 3.8 l]. Radioactivity of the vials was measured at 4°C in a model 500 D Packard scintillation counter.

Inhibition of migration. The cell migration technique used has been described in detail by Cochran (1971). 10^6 lymph node cells alone or mixed with Daudi or YCAB cells in 100 : 1 and 10 : 1 ratio were pelleted in capillary tubes. The tubes were fixed to the base of 19 mm clear plastic planchettes filled with the culture medium and sealed with a coverslip. The chambers were incubated at 37 °C for 18—24 hr. The areas were assessed by planimetry and expressed by the migration index,

$$\frac{\text{Lymph node cell/test cell mix}}{\text{Lymph node cell alone} \times 1.5}$$

Factor 1.5 is based on the observation that a mixture of non-immune lymph node cells and cells from the lymphoblastoid lines covers an area which was on the average 150% that of the lymph node cells alone.

Lymphocytotoxicity. The in vitro lymphocytotoxicity test was performed in the microcytotoxic assay [39]. A 9 and MBG target cells were incubated in humidified 5% CO_2 atmosphera. After 24 hr the culture medium was changed and immunized, control Freund's adjuvant sensitized, and normal cells were added, resulting in different target cell: lymphocyte ratios (1 : 50, 1 : 25, 1 : 12.5, 1 : 6.25, 1 : 3.12). After 48 hr incubation, the lymphocytes were gently washed with BSS and the live adhering target cells were fixed in methanol, dehydrated with 5 N HCl, and stained with diluted Giemsa's dye. In this way only the nuclei were stained, which facilitated counting. Lymphocyte activity was expressed as percentage of the remaining adhering target cells compared to the cell number of target cells without added lymphocytes. For each point, 6—12 parallel wells were set up. As the survival of target cells are sensitive to the environment during cultivation, each test was set up on parallel plates and they were plated differently in the incubator box. In each plate, normal and immune lymphocytes were present.

For detecting lymphocytotoxicity, the ^{51}Cr release technique was performed on Microtest plate II (Falcon Plastics, Los Angeles). Daudi and YCAB target cells were labelled with ^{51}Cr (Sodium chromate, 1 mCi/mMol, The Radiochemical Centre, Amersham). The labelled target cell suspension was set up at a concentration of 5×10^4 0.1 ml in culture medium). Lymph node lymphocytes from immune and normal guinea pigs were suspended in concentrations of 2.5×10^6; 1.25×10^6 . . . till 0.15×10^6/0.1 ml medium. 0.1 ml of different dilutions of lymphocytes with 0.1 ml of target cells was added into each well. The plates were incubated in 5% CO_2 at 37 °C for 24 hr. For determination of the lymphocytotoxic effect, the cell suspension was obtained by rinsing each well with 0.6 ml BSS. After centrifugation, activity of supernatant and pellet was counted in a Packard autogamma spectrometer. ^{51}Cr release was calculated from the total c.p.m. of samples and expressed in percentual values.

Serum antibodies. Antibodies reacting with Daudi cells were detected using the indirect immunofluorescence method.

The serum was tested for cytotoxicity using A 9 cells. The cells were therefore incubated with inactivated serum without complement in 5% CO_2 atmosphere at 37 °C for 48 hr.

Enhancing antibody or factor was investigated in two different ways.

1. In 37 °C water bath for 30 min. From this suspension, 1×10^5 Daudi cells were injected intracutaneously into immunized guinea pigs. A control test was done at the same time with the same number of Daudi cells but without incubation with the serum dilution.

2. The blocking activity of the serum of immunized guinea pigs was investigated by means of the Takasugi and Klein method, described in detail elsewhere [39].

Results

Skin test. Tests were carried out on different animals on the day of sensitization and 2, 4, 6, 8 and 10 days later. Intensity of the reaction was dependent on the number of cells injected and the time which had elapsed since immunization. 1×10^6 cells produced the strongest result, whereas 1×10^3 cells induced a weak reaction solely on day 4.

With 1×10^6 human cells (Daudi) the skin test became positive on the 4th day and showed a maximum on the 6th day (Fig. 1). The skin test was not specific for the human cells since positive reactions were obtained with mouse cells also (YCAB). A skin reaction did not appear in animals which had received MEM of Freund's adjuvant alone. Control tests involving the intracutaneous injection of Freund's adjuvant, MEM or FCS alone, were essentially negative in immunized animals, although occasional tests showed a mild (5 mm ∅) reaction.

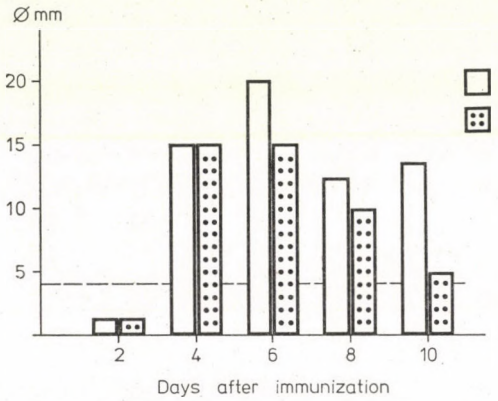

Fig. 1. Cutaneous test in guinea pigs immunized with human lymphoblastoid cells at different times after sensitization. The test was performed with 10^6 human (Daudi) □ and mouse (YCAB) ■ cells. Scores of reaction are indicated by the diameter of erythema and induration. The time indicates the injection. Evaluation was made two days later.
- - - - - - = cutaneous test performed with culture medium (MEM), FCS, in sensitized animals and performed with Daudi and YCAB cells in control animals injected with Freund's adjuvant

The intensity of the reaction to human and mouse cells was similar except after 10 days when the mouse cells induced no reaction but reactivity to the Daudi cells persisted.

Antigenic stimulation of lymphocytes. On the 6th, 8th and 10th days after immunization the lymph nodes were enlarged and firm. This was apparent also in the guinea pigs stimulated with Freund's adjuvant.

Thymidine incorporation of cells from sensitized lymph nodes, in the absence of added specific stimulant cells, increased from day 2 to day 6 (Fig. 2). This is obvious from the values observed on the 3rd and 4th days of incubation. The values were of the same magnitude as those for PHA stimulated control cells. On the 10th day, thymidine incorporation returned to normal levels.

The PHA effect on cells from immunized animals was stronger than in the controls; the difference was not significant statistically (Fig. 3).

Lymph node cells from normal guinea pigs were not influenced by the presence of Daudi, YCAB or CBA cells (Fig. 4). The cells from immunized

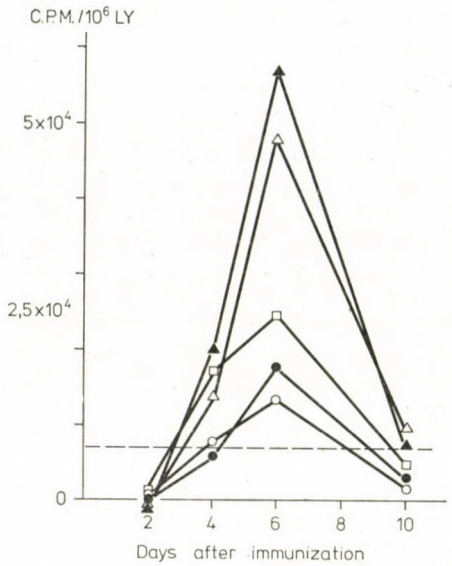

Fig. 2. ^{3}H-thymidine incorporation during 24 hours by the lymph node cell population, at different times after immunization in absence of antigen. The isotope was added; 1 ○ - -, 2 ● - -, 3 △ - -, 4 ▲ - -, 5 □ - - days incubation. - - - - - - c.p.m. of lymph of cells of guinea pigs immunized with Freund's adjuvant. The values are corrected to the c.p.m. obtained for non-immune guinea pig lymphocytes

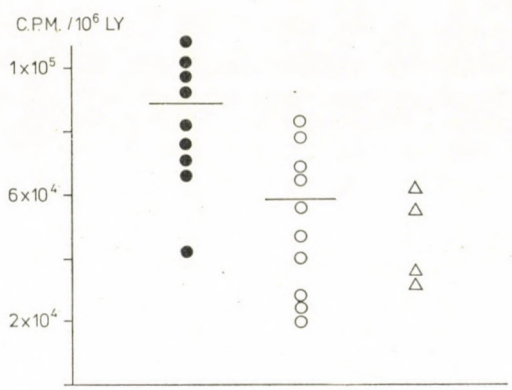

Fig. 3. PHA induced incorporation of ^{3}H-thymidine of lymph node cell populations. The values represent the increase of incorporation above the controls. Lymph node cells from immunized ●, untreated ○, Freund's adjuvant treated △, guinea pigs

guinea pigs were stimulated by exposure to Daudi cells. This effect showed a peak 4 and 6 days after immunization and after 4—5 days of mixed culture. An indication of stimulation was apparent after 1 day and rather strong after 2 days of incubation. Stimulation of these cell populations is thus effected more rapidly than of cells removed before or after that period.

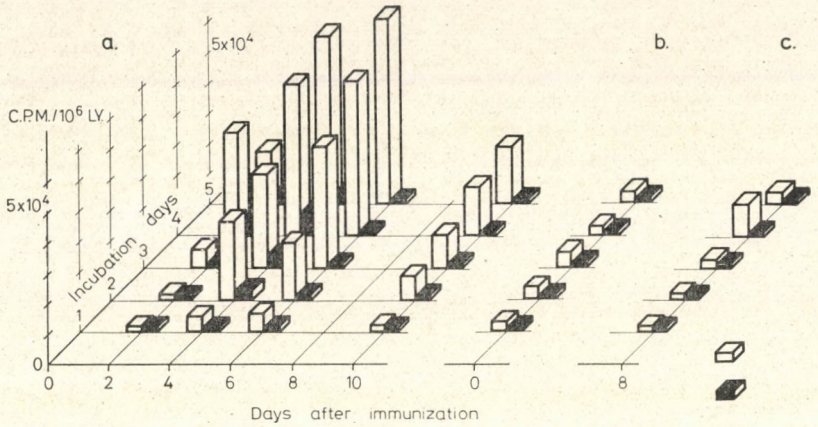

Fig. 4. Incorporation of ^{3}H-thymidine of lymph node cells attributed to the presence of Daudi *Ma*, or YCAB, CBA mouse cells *aM*, related to the time after immunization with Daudi cells. ^{3}H-thymidine was added during 24 hours and after different times of incubation. The c.p.m. values represent the difference of incorporation between the mixed cultures and the lymphocyte cultures. Columns "A" represent the c.p.m. values of sensitized lymph node cells after immunization. In each test, lymphocytes from non-immunized animals were included; the mean results are indicated in columns "B". The c.p.m. columns "C" are the values for lymphocytes obtained from animals treated with Freund's adjuvant

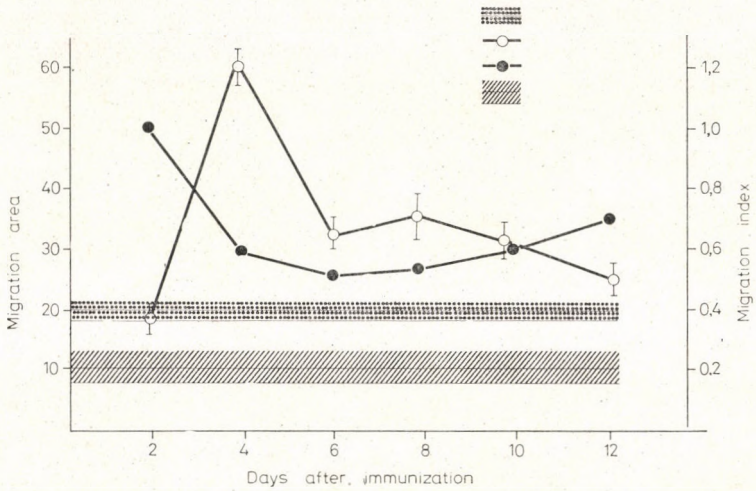

Fig. 5. Migration areas and migration indices of 3×10^6 lymph node cells from immunized guinea pigs. Mean migration area of lymph node cells from animals treated with Freund's adjuvant ⁘. Migration area of lymph node cells from immunized animals —○—. Mean migration area of cells from non-immunized animals ———. Migration index of lymph node cells of immunized animals mixed with Daudi cells in 10 : 1 lymphocyte: Daudi cell ratio —●—. Vertical bars and covered areas indicate standard error of the mean

The lymph node cells of the 4th day were only stimulated by YCAB or CBA spleen cells after 3—4 days subsequent incubation (Fig. 4), but the peak effect amounted to not more than $0.8—1.4 \times 10^4$ c.p.m.

The cells harvested 8 days after treatment with Freund's adjuvant responded weakly to the presence of Daudi cells. The peak response occurred after 4 days mixed culture (c.p.m.: 1.1×10^4; Fig. 4).

Migration studies. Cells from non-immunized guinea pigs migrated poorly (mean area, 11 ± 5.6; range 3—21). Those from immunized animals, were more active (mean migration area, 39 ± 22.2; range 20—27). Maximum values were obtained with cells collected 4 days after immunization (Fig. 5). Motility then declined but 12 days after immunization the migration areas were still significantly greater than those of non-stimulated lymph nodes ($P < 0.01$). Animals immunized with Freund's adjuvant alone yielded lymph node populations on the 6th day after immunization which had a significantly greater migratory capacity than that of untreated animals ($P < 0.01$), but intermediate between the immune and untreated groups (mean migration, 20 ± 1.9; range 19—23).

Contact with the antigens used for immunization (Daudi cells) was inhibitory at 1 : 100 and 1 : 10 and to an extent which varied with the interval between immunization and examination. Maximum depression was found on the 6th day after immunization.

Contact with mouse cells (YCAB) was alone inhibitory but only on days 6 and 8.

Lymphocyte cytotoxicity. Registration of specific cytotoxic activity was attempted against ^{51}Cr labelled Daudi cells. Acitivity was first detected on the 4th day after immunization. It increased in strength on the 6th day (Table I).

Table I

Lymph node cell cytotoxicity on human (Daudi) and mouse (YCAB) target cell lines from immunized guinea pigs on different days after sensitization with human lymphoblastoid cells measured by the $^{51}Chromium$ release technique

Days after immunization	Target cell lines			
	Daudi		YCAB	
	1 : 12+	1 : 25	1 : 12	1 : 25
2	n. t.=	n. t.	n. t.	n. t.
4	0.12*	0.27	0.00	0.00
6	0.34	0.30	0.00	0.08
8	0.04	0.00	0.07	0.05

+ Target cell: lymphocyte ratios.
= Not tested.
* Cytotoxic index (Brunner et al. 1968). Each index represents the values of lymph node cells pooled from 4 immune or 3 normal guinea pigs. In this experiment, spontaneous ^{51}Cr release was less than 40%.

On the 8th day it decreased. Cytotoxicity was exerted at 1 : 1, 25 : 1 lymphocyte target cells. Unfortunately, later the target cells had a high spontaneous release and therefore no data are available concerning the subsequent events.

The cytotoxic effect of immune lymphocytes on non-specific target cells (A9 and MBG) using the Takasugi—Klein method, was different. In the case of A9 target cells a significant lymphotoxic activity was demonstrable from day 4, but this did not appear with MBG target cells (Table II).

Table II

Lymph node cell cytotoxicity on A9 and MBG mouse target cell lines from normal and immunized guinea pigs on different days after sensitization with human lymphoblastoid cells

	Days after sensitization	Percentage survival of target cells+				
		1 : 50=	1 : 25	1 : 12.5	1 : 6.2	1 : 3.1
A9 target cells normal*	—	42.1±8.8**	59.8±12.3	65.5±10.7	72.2±13.8	74.9±13.2
Immunized	2	36.8±8.0	59.5± 9.8	75.0±14.9	79.5±11.8	82.0±19.4
	4	16.8±9.1	30.6±10.2	47.7±10.3	60.8±15.4	—
	6	19.8±3.8	34.5± 7.2	57.5± 7.2	68.3±11.6	83.4±10.6
	8	16.3±5.2	32.3± 5.9	41.0± 8.1	66.5±15.2	73.5± 8.1
	10	8.4±2.5	34.6±12.1	69.1±13.2	72.9±22.7	85.3±13.2
	12	0.2±0.5	24.7±17.0	41.3±10.7	55.8± 9.5	—
MBG target cells normal*	—	86.6±7.8	90.3±10.7	94.0± 5.6	97.6±11.2	103.3± 8.6
Immunized	4	78.2±9.2	69.1±12.3	88.3± 7.2	102.4± 6.8	105.8± 8.2
	6	85.2±6.6	88.7± 7.3	90.1± 8.1	94.4±10.7	103.4± 7.2
	10	84.9±8.3	97.2± 5.8	98.0± 8.6	92.6± 7.2	102.3± 6.0

+ 100% survival = the number of target cells after 48 hours incubation without lymphocytes.
= Target cell: lymphocyte ratios.
* Unimmunized.
** Standard error of the mean.

Serum antibodies and enhancing factor. Using the indirect immunofluorescence technique, antibodies reacting with Daudi cells were detected on day 6 after immunization. 12 days after immunization the serum too agglutinated the Daudi cells. The sera were tested for complement-independent cytotoxicity. Normal serum at 1 : 10 dilution reduced the survival of target cells to 60%. Serum was toxic only from day 10 and 12 and reduced the survival of target cells to 40%. In the same serum samples, enhancing or protective antibodies were not revealed by either method (blocking of in vivo cutaneous test or blocking in vitro lymphocytotoxicity).

Discussion

Contrasting allogeneic systems mixed xenogeneic lymphocyte culture do not show stimulation when non-primed lymphocytes are used [22, 27, 29, 41, 42]. Some species combination are exceptional, e.g. rabbit lymphocytes react

better to xenogeneic (human) than to allogeneic cells [11, 24]. No induction of DNA synthesis was obtained in our mixed culture system when human or mouse lymphoblastoid lines were used as stimulators for guinea pig lymph node cells unless they had originated from immunized animals.

The lack of stimulation by both the mouse and human cells lines is of interest because in several reported experiments cells of lymphoblastoid line were capable of inducing DNA synthesis in the peripheral lymphocytes of the cell line donor [17, 25]. This is believed to reflect an antigenic disparity between the cultured and the autologous cells. No evidence was found for the presence of a soluble blastogenic factor. The lack of such an effect in the xenogeneic combination in our experiment would also indicate that the mechanism does not operate through non-specific factors.

After immunization the lymph node cells responded with high DNA synthesis when confronted with the antigenic cells. Reactivity was highest in the cultures set up on days 4 and 6 after immunization. Thereafter, the stimulability of sensitized lymphocytes decreased but was still marked on day 10. Similarly to other systems, the kinetics of lymphocyte transformability paralleled that of the skin test [3, 23, 28, 30, 36, 45].

Induction of DNA synthesis was highly specific, as it was not possible to stimulate with mouse cells, except slightly at maximum reactivity. This is in accordance with results obtained with hapten-protein conjugates [21, 34, 37, and indicatesithat receptor site of the lymphocytes triggering for DNA synthesis is extremely sensitive for antigenic determinants.

The skin test, since it could be elicited with mouse cells, too, did not show this high degree of specificity.

After immunization the lymph nodes were enlarged and their cells migrated more actively. A number of lymph node cells were transformed, as shown by both an elevated DNA synthesis in vitro and by the greater proportion of blast cells [9, 10]. This reflected the effect of antigen in vivo and could be maintained by carrying over the antigen in some form into the culture. Similar results were reported for ovalbumin-immunized guinea pigs [21]. As guinea pig lymphocytes have a low spontaneous transformation rate [8], the effect is certainly the consequence of immunization. We also found that cell populations with a high spontaneous transformation rate were stimulated more efficiently by PHA. Similar results were reported for guinea pigs immunized with PPD in Freund's adjuvant [14]. These results may indicate that in the early stage of the immune response, a population of activated lymphocytes may be present in the lymph nodes, which can be easily be triggered for further proliferation even by non-specific mitogens.

Stimulation in human mixed lymphocyte cultures shows a good correlation with the genetic relationship of the two lymphocyte partners [2, 13]. However, primary sensitization in vitro does not always correspond to the

genetic background [35]. In view of the results showing a high specificity of sensitized lymphocyte populations in antigen-induced DNA synthesis and the quantitative differences seen in the responses to allogeneic or xenogeneic cells, one has to assume either a substantial difference in the number of antigen reactive cells or a difference in the events leading to stimulation in vitro.

The development of migration inhibition of lymph node cells by contact with specific antigens closely paralleled the development of the cutaneous reaction. The basis of this agreement may partly be determined by the humoral migration inhibition factor which, when injected separately, was shown to cause hyperaemia and induration [5]. The skin test migration inhibition was not entirely specific on days 6 and 8, since contact with mouse cells (YCAB) also caused an inhibition of migration. There was a slight discrepancy between our results and those of other authors using other antigens in which migration inhibition was recorded with some delay when compared to the skin test [32]. Non-specific effects in the migration inhibition test were also seen by Henney and Nordin using BSA and HSG—DNP antigens in the early stage of delayed hypersensitivity [20].

The cytotoxic effect of lymphocytes was also apparent on day 4 as compared to the other in vitro test and to the skin reaction. Using soluble antigens (picric conjugates of ovalbumin), Ruddle and Waksman found a good correlation between the skin test and in vitro lymphocyte cytotoxicity [33]. It may be of interest that non-specific cytotoxicity was evident against A9

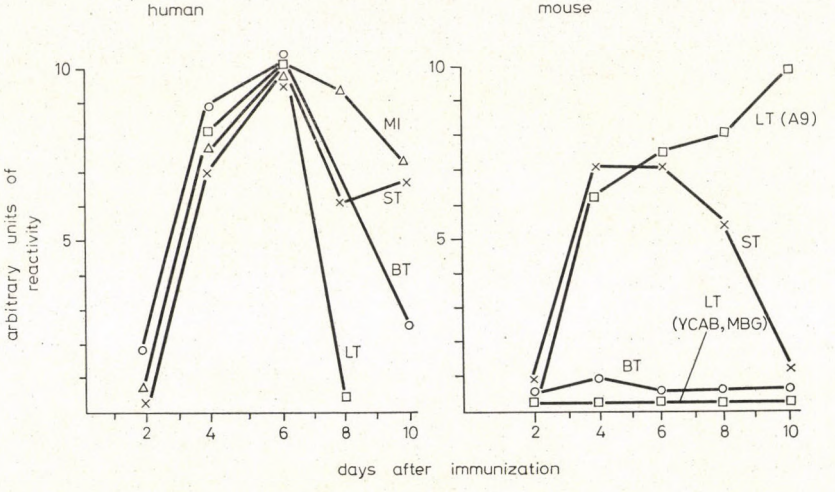

Fig. 6. Kinetics of changes in reactivity of skin test (- - - - x ST), in vitro blast transformation (— — ○ BT), migration inhibition (— — △ MI), lymphocyte cytotoxicity (——□ LT) elicited by HUMAN (Daudi) and MOUSE (YCAB, A9, MBG) antigens during the early phase after immunization with human cells in guinea pigs. The different parameters are on arbitrary scale. The highest value for each parameter was designated arbitrarily as 10 and the other values were expressed in relation to this

target cells, and not against the MBG cells. This fact may suggest that the effect is not strictly cell-mediated as A9 cells are standard targets on which the cytotoxicity of the humoral factors produced by immunoactive or stimulated lymphocytes are demonstrated [19, 40]. This was supported by our finding of cytotoxic antibody or factor in the serum of immunized animals which effected growth of A9 cells. The serum containing specific antibodies had no detectable antibodies or factor which would have protected the target cells against lymphocyte activity.

The strict specificity of the in vitro stimulation of DNA synthesis confined only to the immunizing antigen (human cells), contrasted thus the skin reaction in vivo and the migration inhibition or cytotoxicity in vitro when mouse cells were used (Fig. 6). The positive reaction in the latter case was thus not necessarily coupled to the stimulatory effect of their antigen (cross reacting?) but may have been determined by the behaviour of the transformed lymphocytes in the population upon confrontation with the mouse cells. As mentioned before, this population responded slightly better even to PHA.

Thus, in the early period after immunization, "non-specific" reactions were recorded which were not accompanied by further lymphocyte proliferation. These reactions may be elicited by humoral factors released after confrontation of the lymphocytes with the "non-speficic" antigenic target cells.

*

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THE ORIGIN OF ANAEMIA IN TROPICAL SPLENOMEGALY SYNDROME

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Thirty patients with the diagnosis of tropical splenomegaly syndrome were investigated in Ghana. The patients had low haematocrit and low WBC and platelet counts and the number of reticulocytes and indirect bilirubin were slightly increased. Red cell survival and radioiron studies showed slight haemolysis. IgM immunoglobulin was increased. RBC volume measured with ^{51}Cr tagged erythrocytes was normal but plasma volume assayed with ^{125}I labelled serum albumin was increased. Heat-damaged and ^{51}Cr labelled red cells were used to study microcirculation in the spleen. It was concluded that the low haematocrit was due to the increased plasma volume and the temporary RBC sequestration in the large spleen. In addition, there is a compensated haemolysis.

Tropical splenomegaly syndrome or "big spleen disease" refers to a symptom complex consisting of massive splenomegaly, anaemia and no underlying disease or direct agent which could explain the splenic enlargement. Recently, an increased IgM concentration has been found in peripheral blood and there is a strong support for the view that tropical splenomegaly syndrome is an abnormal and unusual reaction to malarial infection. The relevant facts and theories about the syndrome have been summarized [24] and most of the cases have been reported from areas where malaria is endemic, thus from Senegal [5], Madagascar and Ivory Coast [6], Uganda [19, 29, 13], Zambia [18], Ghana [27, 30] New Guinea [25], Nigeria [8, 36, 31] and in Rwandans living in Uganda [40].

This and other findings point to the important role of malaria in the development of splenomegaly. In this study we are presenting data on the patomechanism of anaemia in the tropical splenomegaly snydrome.

Material and Methods

Thirty patients with tropical splenomegaly syndrome were selected at the Department of Medicine, Korle Bu Teaching Hospital, Accra. The criteria of diagnosis were a very large spleen and no primary cause of the splenomegaly. Falciparum malaria parasites were

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occasionally found in the blood of both the controls and the patients. This is not, however, an unusual finding in individuals living in a hyperendemic area and does not imply clinical malaria. Malaria, like other protozoan diseases, differs from bacterial infections in that the causative parasites do not disappear, but remain in the body where a balance is established between the resistance of the organism and the inherent tendency of the parasites to increase [18a]. All patients were hospitalized and subjected to clinical and laboratory investigations. To serve as controls, patients were selected who had normal haematological values, a spleen of normal size and no disease which would have affected splenic function.

Routine haematological investigation included PCV, Hb estimation, WBC, platelet and reticulocyte counts, and tests for direct and indirect bilirubin [16], plasma volume with ^{135}I tagged serum albumin and RBC volume with ^{51}Cr tagged red cells [21] was performed. Red cell survival was determined following RBC volume estimation. Radioiron studies were carried out [17], and radioactivity over the spleen, liver, heart and sacrum was measured with a 2.54×17.4 cm sodium iodide crystal. Heat treatment and labelling of red cells were carried out according to a method described previously [30]. To summarize the technique briefly, blood was taken into heparin and tagged with ^{51}Cr, this was followed by incubation of the red cells in a water bath ($49.5\,^{\circ}C \pm 0.5$) for 30 minutes. After incubation the cells were washed twice in saline and resuspended in the patient's own plasma. The suspension was reinjected within 30 seconds; the reinjected volume varied between 5 and 8 ml. Blood was taken from the opposite cubital vein at 3, 10, 20 and 40 minutes for measurement of radioactivity.

Radioactivity over the spleen and liver was recorded continuously using a twin probe detector which was used also for isotope renography. One detector was placed over the spleen between the mammillary and anterior axillary lines, the other over the liver, in the anterior axillary line. Care was taken to place the detectors on those parts of these organs where vascularization is maximal, i.e. to avoid the edges. A forearm counter was used for measurements of the circulating radioactivity in the arm without venipuncture.

IgM immunoglobulin was measured by radial immunodiffusion using immunoplates (Behringwerke) and Standard Human Serum (Behringwerke) for calibration.

Results

Splenomegaly. In the majority of the patients, the lower end of the spleen could be found at the umbilicus or lower, not infrequently near the symphisis. The liver was enlarged in almost every case; distended veins over the abdomen were not present in any of the patients.

Age and sex distribution (Table I). The incidence was almost equal in both sexes and mean age was around 36 years in both groups, indicating that predominantly young adults were affected.

PCV, WBC, reticulocytes and platelets (Fig. 1).

Anaemia was present in almost every case. The majority of the patients had PCV values between 20 and 30%. The number of WBC was decreased in two thirds of the cases whereas the platelet count gave low values in every case

Table I

Sex	No.	Age, mean (years)	Age, range (years)
M	17	36.9	15—67
F	13	35.9	18—55
Total	30	36.4	15—67

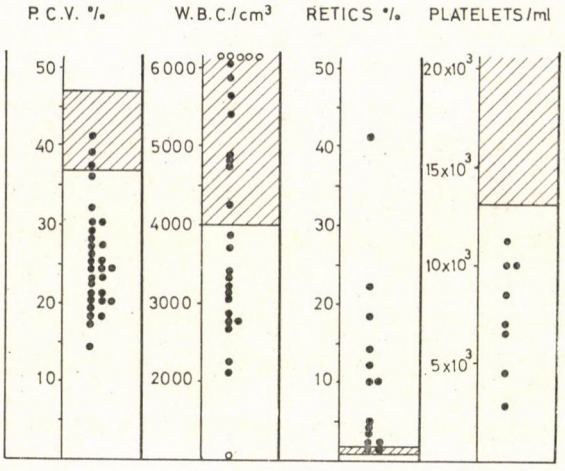

Fig. 1. PCV, WBC, reticulocytes and platelets in patients. Shaded area: normal range

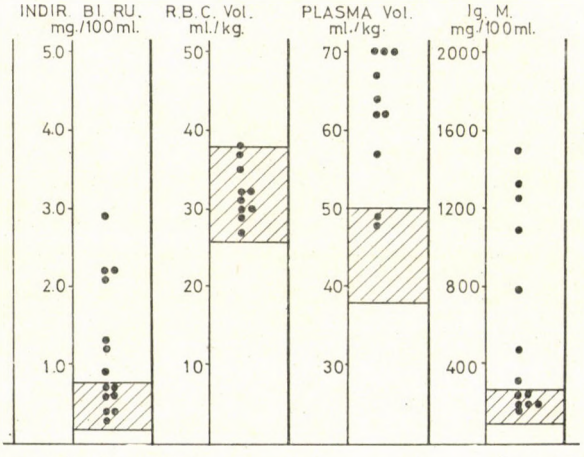

Fig. 2. Indirect bilirubin, RBC volume, plasma volume, and IgM in patients

where it has been estimated. The reticulocyte count showed a moderate increase.

Indirect bilirubin, RBC and plasma volume and IgM concentration (Fig. 2).

Indirect bilirubin was elevated in about half of the patients. In ten cases where the RBC volume was measured, the results fell within the normal range. However, there was a striking increase of plasma volume, the average being 61.4 ml/kg, whereas none of our controls had a value higher than 50 ml/kg.

IgM was increased in more than half of the cases investigated. In some patients it was four to five times higher than the upper limit of healthy young Ghanaians (RINGELHANN, unpublished).

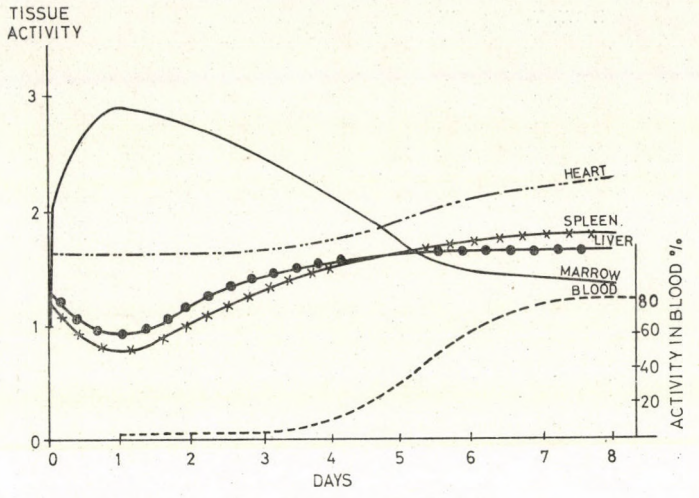

Fig. 3/a. In vivo uptake of radioiron in the normal subject. Radioactivity after five days is highest over the heart. There is a rapid and efficient utilization of radioiron for Hb production

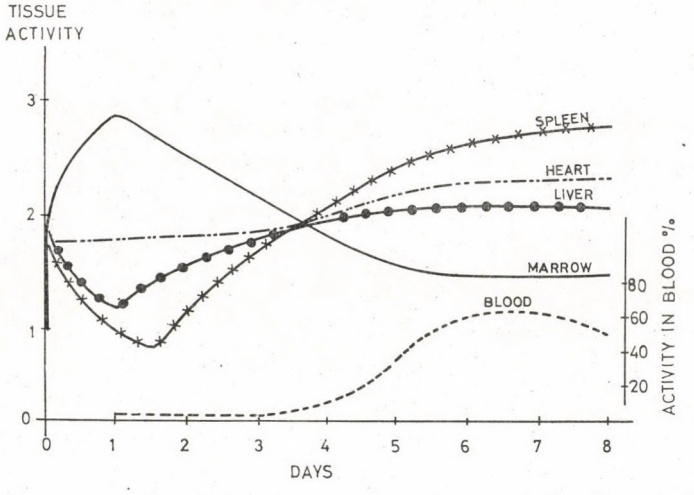

Fig. 3/b. In vivo uptake of radioiron in a patient with tropical splenomegaly syndrome. Utilization of radioiron is less efficient, and iron is deposited in the spleen and liver

Red cell survival and uptake of radioiron in vivo (Figs 3a and 3b).

Red cell survival was measured in four patients; the half life values were 20 days (2 patients), 21 and 25 days. The normal range in our laboratory is 26—35 days.

In vivo estimation of activity after injection of ^{59}Fe labelled plasma transferrin was done in five patients. In four cases the count over the spleen was highest after the 6th day; in the controls, the heart count was highest at

this time. Utilization of iron for Hb production was slightly below 80 %, which is the lower limit of the normal range.

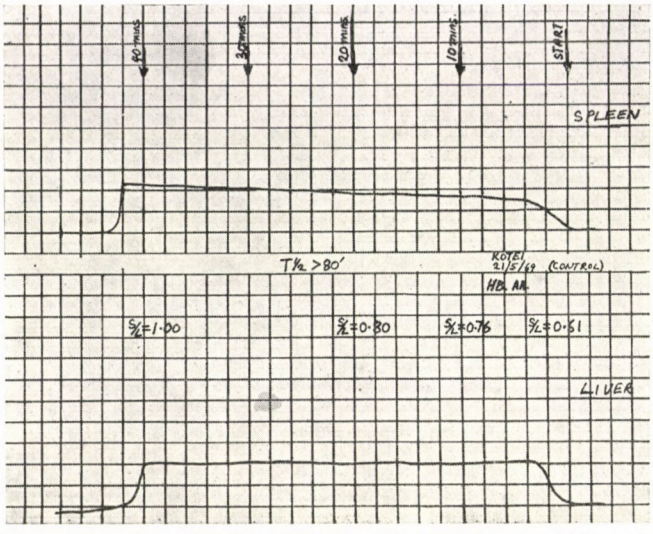

Fig. 4/a. Continuous recording of radioactivity uptake in spleen and liver of a normal subject following injection of Cr^{51} labelled heat-damaged cells

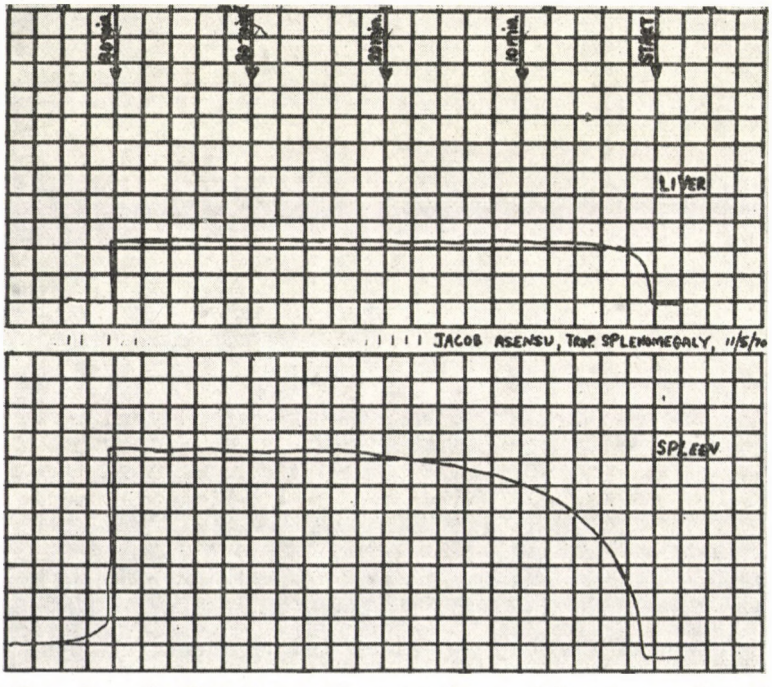

Fig. 4/b. Recording of radioactive uptake over spleen and liver in a patient with tropical splenomegaly. Note the difference in splenic uptake. (Heat-treated cells)

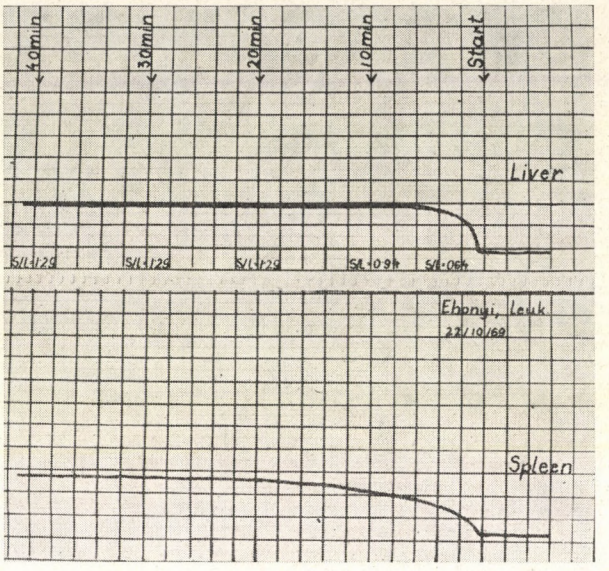

Fig. 5. Continuous recording over liver and the large spleen in a patient with chronic lymphatic leukaemia. (Heat-damaged cells)

Radioactivity over the spleen and in peripheral blood (Figs 4a, 4b and 5) In the normal control individual the injected heat-damaged and ^{51}Cr labelled red cells disappear soon from the circulation and radioactivity over the spleen increases parallel with the disappearance of these cells. In tropical splenomegaly syndrome the disappearance was fast with an increased splenic uptake. The increase of radioactivity over the liver in controls lasted about 3 minutes, then the curve levelled off. In contrast, uptake over the spleen showed a fast increase lasting for about 4 minutes, and this was followed by a slower but continuous rise up to 40 minutes. In tropical splenomegaly syndrome the uptake curve over the liver was similar to that of the controls, and the shape of the splenic curve was also similar, but much greater uptake is rated. In the normal controls, the ratio of splenic and liver count rates was 1.0 while in tropical splenomegaly syndrome the ratio was 3.0. Nevertheless, in one healthy individual selected as a control case we found a fast disappearance of the heat-treated ^{51}Cr tagged red cells, and the spleen-liver ratio was 2.0.

An uptake curve over the spleen and liver in a patient with chronic lymphoid leukaemia resembled that observed in the controls, though the patient had a very large spleen.

Analysis of the uptake curves (Figs 6 and 7). Plotting on log linear plot the difference between the uptake at any given time and the final equilibrium value for the uptake showed two exponential components which could be separated by simple graphical curve peeling. The two components had half times of approximately 1.2 and 6.1 minutes, respectively.

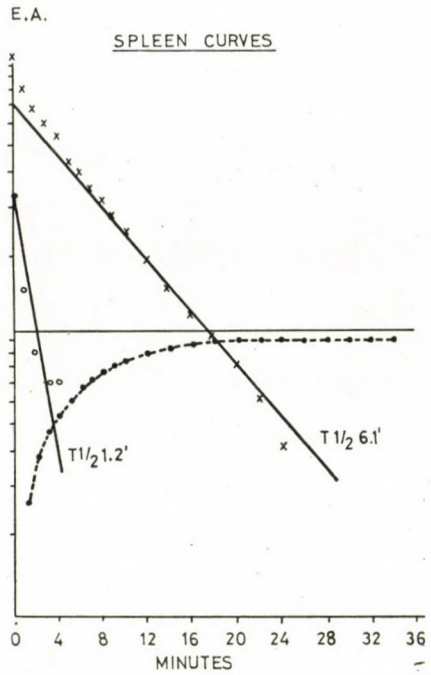

Fig. 6. Radioactivity in spleen of a patient with tropical splenomegaly plotted on a semilogarithmic paper, showing two phases of the uptake. (Heat-damaged cells)

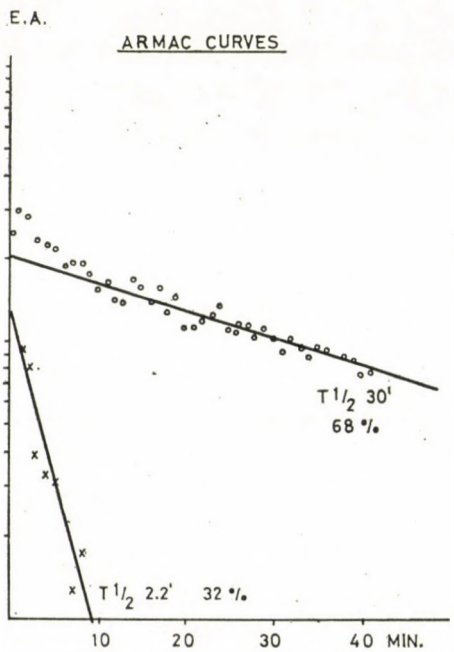

Fig. 7. Radioactivity in the arm of a patient with tropical splenomegaly syndrome, plotted on semilogarithmic paper, showing two phases of the uptake. (Heat-damaged cells)

The half-life of the fast component on the arm curve was about 2.2 min, representing 32% of the total radioactivity. Although there was a reasonable agreement for the short half times in splenic uptake and the disappearance of radioactivity in the arm, there was no agreement for the long ones, the slow component in the arm curve having been 30 minutes, so this figure which represents 68% of the total radioactivity did not agree well with the splenic uptake curve.

Discussion

The haematocrit was low in all but three of our patients and its degree varied between moderate and slight. A similar degree of anaemia was found by other workers [29, 22, 25, 27]. Reticulocytosis, raised indirect bilirubin, shortened RBC survival and the pattern of organ ^{59}Fe uptake implied some premature lysis and increased red cell production, pointing to an anaemia of haemolytic character. Reticulocytosis and a shortened red cell life span were found in tropical splenomegaly syndrome in New Guinea [25], although not in the cases investigated by French authors [22], nor in Uganda where the reticulocyte count ranged between 1 and 4% in 15 patients except one [29]. Despite the low haematocrit and the laboratory findings indicating haemolysis, the red cell volume was normal, and in 8 out of 10 patients the plasma volume was expanded. From the normal red cell volume we may infer that the haemolysis was compensated. This compensated haemolysis may have accounted for the increased medullary erythropoiesis which we have seen in some patients. No thorough morphological investigation of erythropoiesis was carried out in this study. The mechanism which could account for the low haematocrit is haemodilution and this has been found in splenomegalies of various origin [12, 20, 23, 3, 34, 4, 37, 25, 1]. Splenectomy leads to a complete or partial reduction of the blood volume [20, 1, 25, 37, 7, 29, 14]. The origin of the expanded plasma volume has not been clarified. Extension of the vascular bed and a compensatory increase of plasma volume has been suggested [1, 4]. According to another explanation, changes in oncotic pressure [37] and hypervolaemia related to hyperimmunity against malaria could provoke changes in the plasma volume [25]. Indeed, in our patients the concentration of IgM was elevated, similarly as found by other authors [6, 31, 39, 38, 40]. It is noteworthy that the large spleen in tropical splenomegaly syndrome decreases in patients on long term malaria prophylaxis [36, 31] and the blood IgM level also decreases [31]. It is unlikely, however, that the elevated IgM would have caused a hypervolaemia because this latter also occurs in non-tropical splenomegalies without an increase in IgM.

The normal red cell volume and the low haematocrit associated with decreased WBC and platelet count in our patients implicate an enlarged

spleen for pooling of part of the circulating red cells [23, 15, 9, 10, 1, 2, 28]. The spleen after splenectomy may contain 16—30% of the total red cell mass [1]. The prolonged half clearance time of heat damaged red cells after splenectomy in our previous study [30] adds further support to the theory of sequestration in the spleen.

The continuous recording of radioactivity over the spleen and the frequent measurements over the arm in our experiments offered some insight into the mechanism of splenic sequestration. The first phase of the splenic uptake curve (Fig. 6) had a half time of 1.2 min. and the slower phase, 6.1 min. This type of curve mirrors the entry of ^{51}Cr labelled cells into a two compartmental system [12, 15, 23, 28, 33, 9, 10, 11, 3]. The rapid first phase with a rapid outflow rate corresponds to the direct or 'closed' circulatory system of the red cell pulp in the spleen [35, 26] which is a direct arterio-venous shunt. The second phase with a longer $T^1/_2$ and a less steep slope signals a slow inflow rate and the entry of red cells into the 'open' circulation existing parallel to the closed or direct system. Anatomically the open circulatory system is located in the lymphatic cords (Billroth's cord) and forms a loose filter system composed of macrophages and apertures between them of 3 μ in diameter. The environment of this system puts strain on the cells (hypoxia, lower pH) and the old and faulty cells will be removed [15]. In the normal spleen, a small proportion of the cells is diverted toward the slow compartment, in tropical splenomegaly syndrome (and in some other splenomegalies) this proportion increases and the red cells due to a longer stay amidst hyperactive macrophages are prone to untimely lysis.

The 6.1 min half time of the second phase of the splenic uptake curve indicates a gradual build-up of radioactivity in the spleen, in other words a slow inflow of cells into the open circulation. Owing to the slow outflow rate, circulation in the cords slows down and the cells are trapped there temporarily. One third or one fourth of the circulating red cell mass may be sequestred in the large spleen. The activity curve over the arm shows that 32% of the radioactivity is bound to the first phase, and 68% to the second, slow phase.

Not every large spleen can be assumed to be capable of sequestering red cells. Fig. 5 shows the splenic uptake curve of a patient with chronic lymphatic leukaemia, who had a very large spleen. In this case the malignant growth of the lymphatic tissue probably prevented the expansion of the second compartment. In this context we mention that sometimes it is difficult to differentiate between the tropical splenomegaly syndrome and chronic lymphatic leukaemia in those cases where in the peripheral circulation the percentage of lymphocytes is high [31]. The splenic uptake curve after injection of heat-damaged ^{51}Cr labelled cells may help in establishing the diagnosis.

Finally we have to comment on the slow disappearance of radioactivity on the arm curve in the second phase (Fig. 7). In our experience, after 10

minutes there is no close agreement between forearm count and blood count, the former mostly being higher. This is in contradiction to the finding of FISCHER et al. [10] who found closely corresponding results and on account of this they omitted blood sampling and measure radioactivity only in the arm. At a later stage of the postinjection period, some damaged cells and/or cell fragments may, however, be withheld in the capillaries and this excess radioactivity causes unduly higher arm counts.

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РЕЗЮМЕ

ИЗУЧЕНИЕ СВЯЗЫВАНИЯ МОЧЕВОЙ КИСЛОТЫ ПЛАЗМЕННЫМИ БЕЛКАМИ ПРИ ПОДАГРЕ

Е. ХОЛЛЕНДЕР

Автор проводил отделение связанной плазменными белками и свободной мочевой кислоты методом фильтрования на геле Сефадекс. В контрольной группе 20,3%, а в группе больных подагрой 16,9% общего количества мочевой кислоты оказались связанными. При применении 14 С-2-мочевой кислоты в нормальной плазме было найдено связывание мочевой кислоты белками подобного порядка величины. При подагрическом артрите связанная белками активность меченной мочевой кислоты составила только 46% активности в норме. Плазма больных подагрой, не получавших лечения, способна связывать больше мочевой кислоты, чем плазма больных, лечившихся препаратом Зилорик.

Вопреки более низкому соотношению связывания при гиперурикемических состояниях абсолютное количество связанной, неактивной мочевой кислоты достоверно больше, чем у контрольных лиц. Нормальные плазменные белки вследствие способности к связыванию мочевой кислоты до известной степени способны также тормозить тканевое выпадение мочевой кислоты.

Из фракций плазменных белков, отделенных электрофорезом на бумаге, меченная мочевая кислоты связывается α_2- и β_1-глобулинами.

КЛИНИЧЕСКОЕ И ГИСТОПАТОЛОГИЧЕСКОЕ ИЗУЧЕНИЕ ЗАБОЛЕВАНИЙ ПОЧЕК У ЧЕЛОВЕКА

V. Гистологические, иммуногистологические и электронномикроскопические данные ребиопсии

Э. БЕРЕГИ и И. ВАРГА

Сообщаются гистологические, иммуногистологические и электронномикроскопические данные ребиопсии, проведенной у 70 больных заболеваниями почек. Изменения, наблюдавшиеся при повторной биопсии, обсуждаются в распределении на 3 группы: 1. излечившиеся случаи, 2. неизменно существующие процессы, подобные процессу, наблюдавшемуся при первой биопсии, 3. случаи ухудшения. У излечившихся больных под оптическим микроскопом в корешках местами удалось выявить незначительное размножение мезангиальных клеток и мезангиального матрикса, указывающее на протекавший гломерулонефрит. Других изменений не было найдено. Иммуногистологическое исследование привело к отрицательному результату. В электронном микроскопе же на нескольких местах еще удалось доказать слияние отростков клубочковых клеток, в эпителиальных клетках наблюдались липидные капли, а в эндотелиальных клетках — вакуоли. В группе больных с неизменным процессом при ребиопсии под оптическим микроскопом картина была идентичного характера, как и при первой биопсии. При иммуногистологическом исследовании в части случаев наблюдалось исчезновение или уменьшение иммунного депозита, и в то же время на основной перепонке и в мезангии был найден фибрин. Авторы обсуждают связь между наличием фибрина и гломерулонефритом. В электронном микроскопе на многочисленных местах на утолщенной основной перепонке, наряду с иммунным депозитом, видны проточинки. В группе больных с ухудшением процесса в ходе ребиопсии под оптическим микроскопом было выявлено прикрепление и гиалиновое преобразование

клубочковых петель. Иммуногистологическим способом было выявлено такое же изменение, как и в первой группе. Электронномикроскопическое исследование показало размножение мезангиального матрикса и расширение основной перепонки, и, наряду с этим, на основной перепонке и в мезангии наблюдался иммунный депозит, и на основной перепонке — проточинки.

ДЕЙСТВИЕ СЫВОРОТОЧНОГО α_1-АНТИТРИПСИНА НА ПРОТЕОЛИТИЧЕСКУЮ АКТИВНОСТЬ ГНОЙНОЙ МОКРОТЫ

Э. ТАРЬЯН и П. ТОЛНАИ

Авторами был изучен вопрос о том, в какой мере можно тормозить протеолитическую активность гнойной мокроты введением нормальной человеческой сыворотки и таких сывороток, в которых в различной мере понижено количество α_1-антитрипсина.

Было установлено, что последние сыворотки почти или совершенно не понижают протеолитическую активность мокроты, и что степень ингибирования соразмерна содержанию α_1-антитрипсина в сыворотке.

ПРЕССОРНАЯ АКТИВНОСТЬ НАТИВНОЙ ПЛАЗМЫ ПОЧЕЧНОЙ ВЕНЫ

Л. ДЕБРЕЦЕНИ и В. СЕКАЧ

В целях изучения диагностической ценности определения прессорной активности нативной плазмы почечной вены авторы проводили сравнение серийных нефроангиограмм и активности ренина в плазме почечной вены больных гипертонией. Несмотря на то, что пропорционально логарифму активности ренина в плазме почечной вены, в нативной плазме почечной вены была выявлена повышенная прессорная активность, ложноположительные результаты авторов указывают на то, что в индивидуальных случаях измерение прессорной активности нативной плазмы почечной вены не может заменить измерение активности ренина в плазме почечной вены в области доказательства причинной связи между сужением почечной артерии и гипертонической болезнью.

ТОРМОЖЕНИЕ ОБРАЗОВАНИЯ ИММУННОЙ РОЗЕТКИ В КЛЕТКАХ СЕЛЕЗЕНКИ МЫШЕЙ РАДИОКОБАЛЬТНЫМ ОБЛУЧЕНИЕМ МЕТОД ДЛЯ ИЗМЕРЕНИЯ ИММУНОСУППРЕССИВНОГО ДЕЙСТВИЯ ГАММАИЗЛУЧЕНИЯ

Б. ФЕКЕТЕ, ДЬ. СЕГЕДИ, Ю. ПЕТРАНЬИ, П. ГЕРГЕЙ, Г. САБО и З. ДЕЖИ

Авторами было установлено, что облучение всего тела мышей штамма Бальб/с радиоктивным кобальтом в дозе $1/5ЛД_{50}$ представляет собой эффективный ингибитор образования иммунной розетки. Излучение радиоактивного кобальта с одной стороны уменьшает число лимфоцитов селезенки, образующих розетку, а с другой — задерживает образование розетки остальными лимфоцитами.

Наиболее чувствительными к иррадиации являются Т-лимфоциты.

Применение облучения до дачи или в день введения антигена привело к усиленному торможению образования розетки по сравнению с облучением после антигенового стимула.

Результаты, достигнутые методом торможения иммунной розетки, предоставляют новые данные об ингибиторном действии облучения на иммуноциты.

ИЗУЧЕНИЕ ГУМОРАЛЬНОГО ИММУНИТЕТА ПРИ ХРОНИЧЕСКИХ ЗАБОЛЕВАНИЯХ ПЕЧЕНИ

К. ШИМОН, А. ПАТАКФАЛЬВИ, А. ПАР и Ж. МИСЛАИ

Авторами было проведено сравнение поведения гуморального иммунного ответа при различных типах хронических заболеваний печени. Они определяли у 69 больных хроническими заболеваниями печени (хронический активный гепатит, хронический персистирующий гепатит, цирроз печени, жировая печень) величину содержания гаммаглобулинов, РОЭ, тимол и концентрацию трех главных иммуноглобулинов, титры антибактери-

альных противотел. Они изучали также встречаемость аутопротивотелоподобных факторов.

Согласно полученным результатам, при хронических заболеваниях печени, сопряженных реакцией мезенхимы, повышенный гуморальный иммунный ответ представляет собой общее явление. На основе вышеуказанных лабораторных показателей и изучения иммуноглобулинов и противотел можно делать заключение о наличии или отсутствии реакции мезенхимы. При хронических заболеваниях печени, сопровожденных реакцией мезенхимы, наблюдалось прежде всего повышение количества антител против кишечных бактерий, тогда как противотела против некишечных бактерий не показали характерного изменения. Выявление автопротивотелоподобных факторов в сыворотке может оказать помощь при открытии автоиммунных механизмов.

ВЛИЯНИЕ БЕТА-АДРЕНЕРГИЧЕСКОГО ВОЗБУЖДЕНИЯ НА КОРОНАРНОЕ КРОВООБРАЩЕНИЕ ПРИ СЕГМЕНТАРНОЙ ИШЕМИИ МИОКАРДА

Ш. ЮХАС-НАДЬ и ДЬ. ГРОС

Авторы исследовали у наркотизированных собак с открытой грудной клеткой реакцию коронарного кровообращения на дачу изопротеренола (0,1 μг на кг$^{-1}$ мин$^{-1}$ — 1,6 μг на кг$^{-1}$ мин$^{-1}$) в норме и при ишемии миокарда, вызванной сжатием передней нисходящей ветви (RD). Коронарное кровообращение авторы измеряли отчасти способом определения венозного оттока из венечного синуса сердца и отчасти методом теплового клиренса в сегменте желудочка, снабжаемом RD. Было установлено, что бета-адренергическое сосудорасширяющее действие, оказанное на общее коронарное кровообращение, после сжатия RD до некоторой степени снижается, причем, однако, крутость кривых дилятации венечной артерии в зависимости от дозы не показала значительного изменения. Изопротеренол вызвал также в местном кровообращении ишемической зоны расширение сосудов, размер которого было примерно половиной дилятации, измеряемой на том же месте до сжатия RD. Из полученных результатов авторы сделали вывод, что бета-адренергическое возбуждение вызывает, подобно расширению нормальных венечных артерий, также и расширение коллатералей.

АБСОРБЦИЯ И ТРАНСПОРТ БЕЛКОВ ИЗ БРЮШНОЙ ПОЛОСТИ

Г. САБО и З. МАДЬЯР

Абсорбция меченного J^{131} альбумина с диафрагмальной стороны брюшины, серозной оболочки печени и кишок была изучена авторами на собаках. С поверхности печени абсорбция альбумина была примерно в четыре раза более быстрой, чем с поверхности диафрагмы. Из серозной оболочки кишок абсорбции белка не происходит. Около 40% абсорбированных белков проникает непосредственно в капилляры. Лимфатические сосуды, соединяющие крупные кровеносные сосуды на правой стороне шеи, транспортируют в два раза больше абсорбированного белка, чем грудной проток.

ИЗУЧЕНИЕ ВЫДЕЛЕНИЯ НАТРИЯ У ЗДОРОВЫХ ЛИЦ И У БОЛЬНЫХ В РАЗЛИЧНЫХ СТАДИЯХ ГИПЕРТОНИЧЕСКОЙ БОЛЕЗНИ ВЛИВАНИЕМ РАСТВОРА ПОВАРЕННОЙ СОЛИ

Э. ПОЛГАР, Б. КАНЬАР, Ф. ШОМОРЬЯИ и К. КОМОР

Сообщается результат исследований, проводившихся у 112 больных вливанием 5%-ого гипертонического раствора поваренной соли.

В экспериментах с острой нагрузкой солью наблюдались достоверные разницы между натриурезом контрольных здоровых лиц и больных гипертонией.

Достоверная разница была выявлена также между показателями контрольной группы и нормотензивной гиперреакторной (NAT-положительной) группы.

Между реакциями гипертонической и гиперреакторной групп не удалось выявить существенной разницы.

ОТДАЛЕННАЯ ЧРЕЗМЕРНАЯ ЧУВСТВИТЕЛЬНОСТЬ МОРСКИХ СВИНОК, ВЫЗВАННАЯ КСЕНОГЕННЫМИ КЛЕТКАМИ; РАЗВИТИЕ БЛАСТОЗНОГО ПРЕОБРАЗОВАНИЯ, МИГРАЦИИ И ЦИТОТОКСИЧНОСТИ КЛЕТОК ЛИМФАТИЧЕСКИХ УЗЛОВ, А ТАКЖЕ КОЖНОЙ РЕАКЦИИ in vitro

Г. ДЬ. ПЕТРАНЬИ, Е. КЛЕИН, А. Й. КОХРАН, Э. СВЕДМИР и Х. ЯКОБСОН

Авторами было изучено в ранней фазе отдаленной чрезмерной чувствительности, вызванной у морских свинок введением человеческих лимфоидных клеток, развитие активности лимфоцитов in vitro (бластозная трансформация, торможение миграции и цитотоксичность) и возникновение кожной реакции. В регионарных лимфатических узлах иммунизированных животных поглощение 3Н-тимидина лимфоцитами и активность миграции оказались, даже без наличия антигена, повышенными, в частности на шестой день после иммунизации. В присутствии же антигена лимфоциты показали активность уже начиная с второго дня после иммунизации. Кинетика активности лимфоцитов, выявленной в опытах in vitro, была параллельной с протеканием кожной реакции и показала максимальную активность на шестой день. Неспецифическим антигеном (опухолевые клетки мышей) кожная реакция и цитотоксичность in vitro давали положительный, а бластозная трансформация — отрицательный результат. Лимфоциты контрольных, неиммунизированных морских свинок ни в одном опыте не дали положительного результата.

ПАТОГЕНЕЗ АНЕМИИ ПРИ СИНДРОМЕ ТРОПИЧЕСКОЙ СПЛЕНОМЕГАЛИИ

Б. РИНГЕЛХАНН, Х. МИЛЛЕР и Ф. И. КОНОТЕЙ-АУЛЬУ

Авторы исследовали в Гане 30 больных диагнозом синдрома тропической спленомегалии. Они нашли низкие параметры гематокрита, числа лейкоцитов и тромбоцитов, тогда как число ретикулоцитов и величина непрямого билирубина оказались несколько повышенными. Изучение выживания эритроцитов и радиоактивного железа также показало понижение гемолиза. Фракция IgM иммуноглобулинов была увеличена. При помощи эритроцитов, меченных Cr^{51} число эритроцитов не было повышенным, однако объем плазмы, определенный сывороточным альбумином, меченным J^{125}, оказался выше нормы. Для изучения микроциркуляции селезенки, авторы применяли теплоубитые и меченные Cr^{51} эритроциты. Они пришли к заключению, что низкий показатель гематокрита обуславливается совместным эффектом увеличенного объема плазмы и временной секвестрации числа эритроцитов в увеличенной селезенке. Кроме того имеется также компенсированный гемолиз.

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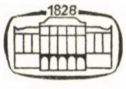

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