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Endocrinology

THE SIGNIFICANCE OF THYROID-STIMULATING ANTIBODY (TSAb) IN THE MANAGEMENT AND PROGNOSTIC EVALUATION OF GRAVES DISEASE

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(Received: January 9, 1984)

Sixty six patients with Graves disease were followed up for the presence of TSAb at intervals of 4 to 12 weeks. Three different assays, the competitive TSH membrane-receptor assay (MRA), the cAMP-assay and the colloid droplet assay (CD) were used in parallel for demonstration of the antibody.

In 17 of the patients TSAb was demonstrable also during remissions. 11 of these patients relapsed within a year. TSAb-positivity decline 1 to 4 months after ^{131}I treatment. In case of persisting TSAb positivity ^{131}I therapy or surgery should be given preference to thyrostatic medication.

In gravidae followed up for TSAb, positivity not only persisted throughout pregnancy but even increased during the last trimester. After delivery all patients relapsed. One of the gravidae died of pregnancy toxicosis, the infant of another woman was thyrotoxic at birth.

In pregnant women who have Graves disease and are positive for TSAb, thyrostatic or surgical treatment is recommended after the 2nd trimester even in cases of minor severity.

Keywords: thyroid stimulating antibody, Graves disease

Introduction

Recent research has not only given closer insight into the role of thyroid stimulating antibodies in the pathogenesis of diffuse thyrotoxic goitre, but has been furnishing increasing evidence of their predictive value as concerns the duration of remissions [3, 4, 6, 9, 13, 21, 22, 26, 29, 30, 32]. It has been found, indeed, that long-term remissions are unlikely to take place unless the TSAb demonstrated earlier has disappeared from the blood, otherwise a relapse within a short time is to be expected. In other words, TSAb positivity in remissions indicates that, in spite of the clinically euthyroid state, the immunological activity of the disease still persists.

The TSAb-assay may thus be regarded as an indicator of the duration of therapy, as well as of the therapeutic lines to be followed in a given case [11, 31].

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Recent investigations relating to the histocompatibility genes have provided some remarkable predictive clues to the responsiveness of the process and to the duration of remission. McGregor et al. [19] studied the TS-immunoglobulin levels in association with typing for histocompatibility genes, after the determination of thyrostatic treatment. The results were found predictive with a 95% accuracy of the further course of the disease. More than 90% of the DR_3-positive patients who had been treated with carbimazole for six months, relapsed within 12 months, whereas in case of DR_3-negativity only 55% relapsed if at the time of drug withdrawal TSAb was demonstrable. On the grounds of these findings, the authors regard HLA-DR_3-positivity as indicative of destructive therapy (^{131}I) or thyroid surgery. A connection between suppressor T-cell activity and positivity for HLA-B8 antigen was found by the present authors [1].

Further information on the relationships in question was sought by a follow-up of 66 patients with Graves disease for the presence of TSAb, in the active as well as in the inactive phase. The intervals between the assays were between 4 and 12 weeks.

Material and methods

52 females and 14 males, aged 18–64 years, were studied. Diagnosis was based on the Crooks-test expressing numerically the global clinical situation and on the following laboratory parameters: T_3U, T_4, FT_4I, ^{131}I-retention curve, TRH-TSH stimulation test, and T_3-suppression test.

For the demonstration of TSAb three methods were employed (Table I):

1. The competitive TSH membrane-receptor assay (MRA)
2. Measurement of the cAMP-content in human thyroid slices (cAMP assay)
3. Study of the intensity of colloid droplet (CD) formation in surviving human thyroid slices (CD-assay).

Since Graves disease in pregnancy involves difficult problems [10, 16] including the role and clinical implications of immune factors which have yet to be clarified, 4 gravidae were also included in the study and followed up from the first month of pregnancy throughout ts entire duration, beyond delivery and post-partum-period, for a total of 12 months.

Table I

Demonstration of human TSAb

a) Competitive TSH membrane-receptor assay (MRA) according to the "radioligand" method of Smith and Hall [28]

b) Measurement of cAMP concentrations of human thyroid slices, according to Onaya et al. [24] and McKenzie and Zakarija [20], with modifications (Bordán et al. [5]).

c) Colloid droplet (CD) formation, i.e. colloid pinocytosis of acinar cells of surviving human thyroid slices, examined by the method of Onaya and Solomon [23] and Onaya et al. [24] modified by Leövey et al. [14].

Results

From among the 66 patients 47 were assayed for TSAb on two occasions, 16 patients on three different occasions, three patients on 4, 6 and 7 occasions, respectively (Table II).

It was remarkable to find that in 17 of the 66 cases TSAb-positivity persisted also during remissions of the disease. The results of all three assays remained positive in 6, of two assays in 7, of one in 4 cases. The results have been analysed according to Table III.

Until completion of the present report 11, that is 64% of the patients relapsed within a year. The average duration of remission had been 6 months in these cases. If thyrostatic or radioiodine treatment resulted in remissions of a year or more, the TSAb-assays became negative within 3 to 4 months (range 1–11 months).

Nineteen of the 66 patients were subjected to radioiodine therapy with doses of 5 to 8 mCi (185 to 296 MBq), this allowed to follow up the response of TSAb in time within a period of 1–10 months. The different TSAb-assays

Table II

Global results of TSAb-assay in 66 cases

Number of cases	Number of assays	Positive		Negative	
			percent		percent
47	2	41	87.2	6	12.8
16	3	16	100	—	
1	4	1		—	
1	6	1		—	
1	7	1		—	

Table III

Persisting TSAb-positivity during remission of Graves disease
(Follow-up of 66 patients)

Assays	Number of cases	Duration months	66 patients percent
MRA + cAMP + CD	6	2-10-8-6-10-5	
MRA + cAMP	3	6-7-5	
MRA + CD	1	3	
cAMP + CD	3	10-3-17	
MRA	2	2-1	
cAMP	1	3	
CD	1	6	
Total	17		25.8

showed a decrease in 24 instances, according to the following distribution: MRA = 9, CD = 8, cAMP = 7 (Table IV). No distinct changes in the initial values were found in 8 cases. (MRA = 6, cAMP = 1, CD = 1) while an increase was noted in 10 cases (cAMP = 5, MRA = 4, CD = 1).

Table IV

Follow-up after ^{131}I-therapy

Assay	Decrease		Increase		No change	
	Values	Time	Values	Time	Values	Time
MRA	21—77	1 month	37—23	1 month	0—4	1 month
	65—91	10 months	90—49	7 months	54—50	6 weeks
	13—30	1 month	51—11	3 months	106—83	3 months
	18—68	6 months	57—22	1 month	171—112	6 weeks
	16—85	6 months			61—62	7 months
	63—87	3 months			16—15	1 month
	14—24	4 months				
	14—33	8 months				
	63—85	4 months				
cAMP	228—100	6 weeks	150—390	1 month	463—450	1 month
	900—500	1 month	125—257	6 months		
	221—185	10 months	455—700	3 months		
	1399—311	7 months	200—285	1 month		
	245—140	6 weeks	200—319	6 months		
	370—310	7 months				
	290—200	1 month				
CD	283—128	1 month	128—311	1 month	100—124	6 weeks
	182—150	1 month				
	380—310	1 month				
	676—476	10 months				
	595—385	7 months				
	394—128	1 month				
	311—162	1 month				
	422—25	4 months				

n = 19

Discussion

On the grounds of the present findings, TSAb-positivity and clinical activity persisting for 6 months or more under thyrostatic treatment is felt to be indicative of therapeutical destruction (^{131}I) or surgical removal of the thyroid. In our experience neither the type of assay which has given the positive result, nor the grade of positivity is of any decisive therapeutic importance. It is, on the other hand, desirable to follow up the cilinical course by parallel

TSAb-assays of different types, so as to avoid false-negative results and to increase diagnostic accuracy. In fact, negativity of a single assay is in our view by no means exclusive of the positivity of either of the other two assays or of both.

Global assessment of the constellation and prediction of the course in a given case would require the additional evidence of histogenetic studies. To form a view on this question we have, however, to wait until further evidence furnished by large-scale studies is forthcoming.

Our observations with ^{131}I-therapy are at variance with earlier results of Pinchera et al. [25] and with more recent reports of Mukhtar et al. [21], McGregor et al. [17], Fenzi et al. [8], Teng et al. [31] and Bech [3], according to whom LATS, MRA and adenocyclase-cAMP increase after ^{131}I-therapy. The general observation of these authors has been that the values rise in the course of the first 1 to 7 months, but gradually decline thereafter until the result had become negative, which is attained in the majority of cases in 9 to 12 months after treatment. It is alleged as the main cause of these findings that radioiodine causes gross damage to the epithelial cells of the thyroid, thus giving rise to a release of antigens which act at first as stimulators of the immune system, including the formation of thyroid-stimulating antibodies. It is further assumed by McGregor et al. [18] that the radioresistant helper T-lymphocytes are capable of stimulating the "non-irradiated immune cells", endowing them with the ability of repopulation and autoantibody formation. It seems obvious that during the first 3 or 4 months following upon ^{131}I-treatment when destruction of the thyroid parenchyma has run its course, the blood stream may be invaded by antigen-components, under the effect of which certain afferent and/or efferent subunits of the immune system are activated or hyperactivated. These considerations prompted us to form separate groups from those 13 patients who had been checked for TSAb during the first 1 to 4 months after treatment. The results are presented in Fig. 1. A decline of the MRA-, cAMP and CD-values was found in 14 instances, an increase or no change in 7 instances each. Since, as it can be seen, we have found a decrease in the majority of the cases, we fail to confirm the findings referred to above. It might be assumed for the interpretation of these results that, in consequence of the destructive process involving the thyroid parenchyma, certain antigen components finding access in larger amounts into the circulation may have bound and neutralized the thyroid-stimulating immunoglobulins in the form of immune complexes [3].

In the 4 gravidae under study thyrotoxicosis was of minor or moderate severity throughout the entire pregnancy, therefore, thyrostatic treatment involving certain hazards to the fetus was dispensed with. Delivery was, however, invariably followed by a deterioration of thyrotoxicosis requiring thyrostatic therapy in all of the cases. All gravidae had been TSAb-positive throughout the entire pregnancy, and it is a point of interest that the TSAb-values

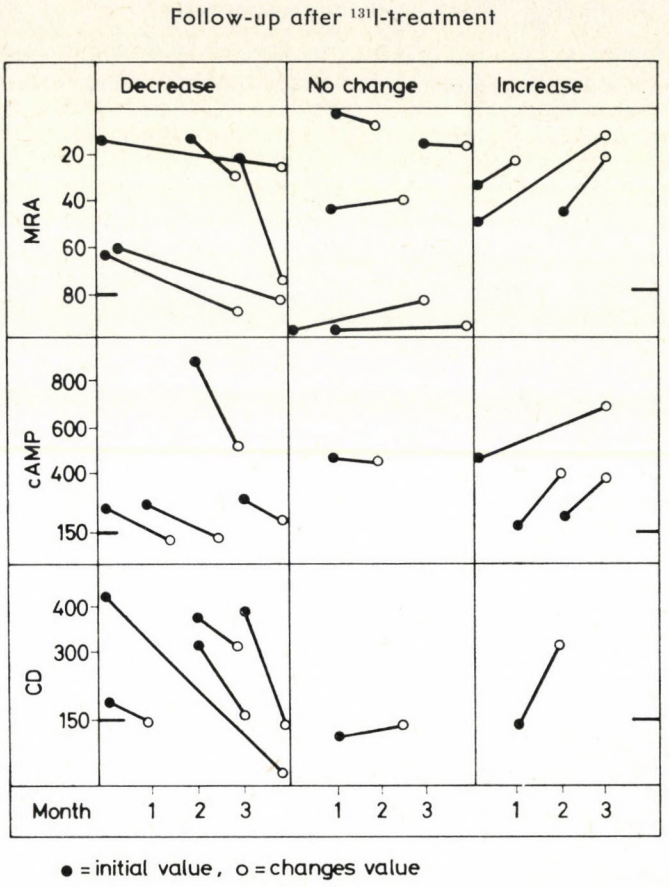

Fig. 1

had increased during the last trimester. From a single exception apart, the other laboratory values were in conformity with these findings (Table V).

In Cases 1 and 3, delivery was smooth, both women gave birth to full-term, healthy infants. On account of the clinical severity, methimazole treatment had to be started immediately after delivery in the first case, and three months later in the other.

The infant of patient J. L. was thyrotoxic at birth, with a pulse rate of 180/min, a T_3U of 1.47 and T_4 283 of nmol/1. Tachycardia improved on beta-blockers and the clinical signs of thyrotoxicosis ceased spontaneously by the end of 2 months. Placental transmission of maternal TSAb has been made responsible for the symptoms [7]. Congenital Graves disease may, however, persist for months or even years [12], in which case not only passive transmission but involvement of immunogenetic factors has also to be considered.

Patient A. I., when 7 months pregnant, developed pregnancy toxicosis of extreme severity with nephropathy and excessive hypertension. Premature delivery of the fetus which had died in utero failed to produce any relief. Owing to renal failure she was maintained on haemodialysis for two months. Development of the full-blown pregnancy toxicosis went hand in hand with deterioration of the hyperthyroid condition, parallel with an increase in the TSAb value. She was started on carbimazole, but died of pulmonary embolism 2 months after delivery.

Table V

Follow-up of pregnants with Graves' disease

		Month					Notes
		1	3	6	9	12	
1. P. Z. aged 23	FT_4I	293	256	222	330	129	Full-term, healthy child
	MRA	31.5	42.6	54.3	39.5	—	
	Th.	Ø	Ø	Ø	Met.	Met.	
2. J. L. aged 27	FT_4I	244	212	244	416	318	Full-term child, born with thyrotoxicosis. $FT_4I = 416$. 2 months later euthyroid
	MRA	35.5	47.4	30	33.5	21.8	
	Th.	Ø	Ø	Ø	Ø	Met.	
3. K. L. aged 32	FT_4I	311	189	280	197	343	Earlier ^{131}I, carbimazol treatment. Full-term, healthy child
	MRA	57	31.6	48.6	26.5	71.4	
	Th.	Ø	Ø	Ø	Ø	Met.	
4. A. I. aged 34	FT_4I	422	468	384	104	—	In the 7th month pregnancy toxicosis. Premature delivery of dead fetus. Renal failure, haemodialysis, fatal pulmonary embolism. Previously on carbimazol
	MRA	57.5	43.7	66.4	29	—	
	Th.	Ø	Ø	Ø	Met.		

Met. = methimazole

The present observations suggest that TSAb-positivity in the course of pregnancy indicates that, unless adequate treatment is given, positivity will persist throughout the entire gestation and increase during the last trimester, which heralds a relapse after delivery. For this reason, TSAb-positivity during pregnancy, even in hyperthyroidism of minor severity, calls for thyrostatic therapy or surgery in the second trimester.

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CONCENTRATIONS OF ANDROGENS AND C_{19}-STEROID SULPHATES IN ABDOMINAL SKIN OF HEALTHY WOMEN AND MEN

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Protein-binding assay and radioimmunoassay were used to determine the concentrations of free androgens and C_{19}-steroid sulphates in suprapubic abdominal skin slices obtained from healthy women and men during appendectomy. It was found that the abdominal skin of the women contained DHA in the highest concentration, followed in decreasing order by And., Δ^4-dione, Δ^5-diol, Test. and DHT. The sequence was the same for the men, except that Δ^4-dione preceded And. There was no significant difference in the concentrations of free androgens in abdominal skin of women and men; only the concentration of Δ^4-dione was somewhat higher in the skin obtained from males ($P < 0.05$).

Of the C_{19}-steroid sulphates, And.-S was found in the highest concentration in abdominal skin of females, followed in decreasing order by DHA-S, Δ^5-diol-S and Test.-S. The corresponding decreasing sequence for the men was DHA-S, And.-S, Δ^5-diol-S and Test.-S. The concentration of DHA-S was significantly higher ($P < 0.001$) in men than in women; in the case of Δ^5-diol-S, only a slight degree of significance could be observed ($P < 0.05$).

Keywords: androgens and C_{19}-steroid sulphates, human skin tissues

Introduction

It is known from previous investigations that numerous free androgen steroids and C_{19}-steroid sulphates can be detected in the lipid film layer covering the human axillary and pubic hair [8, 9, 10]. It is also known that the accessory glands of the human skin, the apocrine sweat glands, excrete a considerable quantity of water-soluble steroids onto the surface of the skin [9, 12]. An intensive androgen metabolism occurs in the skin [4, 5, 6], and androgen receptors have been demonstrated in the sebaceous glands and in the skin [1, 11, 14, 15] and, accordingly, the human skin is nowadays regarded as a typical androgen target organ.

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Abbreviations: (Δ^4-dione) = 4-androstene-3,17-dione; Androsterone (And) = 3α-hydroxy-5α-androstan-17-one; Androstenediol (Δ^5-diol) = 5-androstene-3β, 17β-diol; Dehydroepiandrosterone (DHA) = 3β-hydroxy-5-androsten-17-one; Dihydrotestosterone (DHT) = = 17β-hydroxy-5α-androstan-3-one; Testosterone (Test) = 17β-hydroxy-4-androsten-3-one; Dehydroepiandrosterone sulphate (DHA-S) = 3β-sulphooxy-5-androsten-17-one; Androstenediol sulphate (Δ^5-diol-S) = 5-androstene-3β,17β-diol-3-sulphate; Androsterone sulphate (And-S) = 3α-sulphooxy-5–α-androstan-17-one; Testosterone sulphate (Test-S) = 17β-sulphooxy-4-androsten-3-one

The suprapubic area is the one most exposed from the aspect of hirsutism. It appeared interesting to examine how the concentrations of free androgens and their sulphate esters vary in the suprapubic abdominal skin of healthy women and men. We first elaborated a procedure for the simultaneous determination of free androgens and their sulphate esters in abdominal skin, and with this procedure we studied the concentrations of the said steroids in abdominal skin excised from healthy women and men undergoing appendectomy under general anaesthesia. It was also studied whether a sex difference could be detected in the androgen levels. An account of these investigations is presented here.

Materials and methods

1. Aqueous solutions were prepared with glass distilled water

2. Thin-layer chromatography. The purity of the authentic radioactive and "cold" steroids was checked, and the steroids extracted from skin were isolated on 5×28 cm thin-layer plates prepared with Al_2O_3-G (E. Merck, Type 60/E) and silica gel G (E. Merck, nach Stahl) adsorbents. The following systems were used.

Alumina plates.

TLC-"G": n-hexane — ethyl acetate — absolute ethanol — glacial acetic acid (120 : 130 : 1 : 2, v/v/v/v).

TLC-"C_2": benzene — absolute ethanol (97 : 3, v/v).

Silica gel G plates:

TCL-"3": Chloroform-methanol (97 : 3, v/v).

TLC-"I": absolute ethanol-ethyl acetate-concentrated NH_4OH (50 : 50 : 10, v/v/v).

3. The radioactive steroids used in the study were as follows: [7-^{3}H(N)] dehydroepiandrosterone (spec. act. = 24 Ci/mmol), [1β, 2β-^{3}H]testosterone (spec. act. = 44.6 Ci/mmol), [1α, 2α(n)-^{3}H] dihydrotestosterone (spec. act. = 60 Ci/mmol) and [1,2-^{3}H(n)]4-androstene--3,17-dione. (spec. act. = 46 Ci/mmol) were products of the Radio Chemical Centre (Amerham), while [7-^{3}H(N)]5-androstene-3β, 17β-diol (spec. act. = 20 Ci/mmol), [1,2-^{3}H(N)] androsterone (spec. act. = 40 Ci/mmol) and the ammonium salt of [7-^{3}H(N)]-dehydroepiandrosterone sulphate (spec. act. = 24 Ci/mmol) were obtained from New England Nuclear (Boston). Before use, the free steroids were purified on a 4 g Al_2O_3 column (E. Merck, Broockmann III/IV activity [9] and their purity was then checked on Al_2O_3-G thin-layer in TLC-"G" [9].

4. We prepared [7-^{3}H(N)]5-androstene-3β, 17β-diol-3-sulphate (spec. act. = 24 Ci/mmol) from [7-^{3}H(N)DHA-S by reduction with $NaBH_4$. Before use, the tritiated DHA-S and the Δ^5-diol-3-S were purified on a 1.5 g florisil column (60/100 mesh, Floridin Co., Tallahassee, USA), and their purity were checked on silica gel thin-layer in TLC-"I" [9].

5. The non radioactive authentic steroids were bought from Ikapharm (Ramat-Gan, Israel). Before use, they were purified on a 4 g Al_2O_3 column, and their purity was checked on a thin-layer [9].

6. Subjects and skin samples

The abdominal skin samples were obtained from healthy women and men in the course of appendectomy under general anaesthesia. These individuals did not suffer from any endocrine or other disease. The abdominal skin samples were cleaned from antiseptic and from accessory fat tissues, and were processed within 30 minutes following surgery. The abdominal skin samples used contained both dermis and epidermis.

7. Determination of concentrations of free androgens and C_{19}-steroid sulphates in abdominal skin tissue. The concentrations of free androgens (DHA, And., Δ^4-dione, Δ^5-diol, Test. and DHT) and the most important C_{19}-steroid sulphates (And.-S, DHA-S, Δ^5-diol-3-S and Test.-S) were determined simultaneously in 0.5–1.0 g quantities of abdominal skin.

0.5–1.0 g of the cleaned whole skin tissue was cut into 15–20 μ slices in a cryostat at -20 °C and the slices were left to stand for 24 hours in 10 ml 2 N NH_4OH at room temperature. During this period the mixture was shaken intensively. Subsequently it was centrifuged for 10 minutes at 5000 r.p.m. in a cooled centrifuge, and the supernatant (10 ml) was transferred to a ground-glass stoppered tube (Fig. 1).

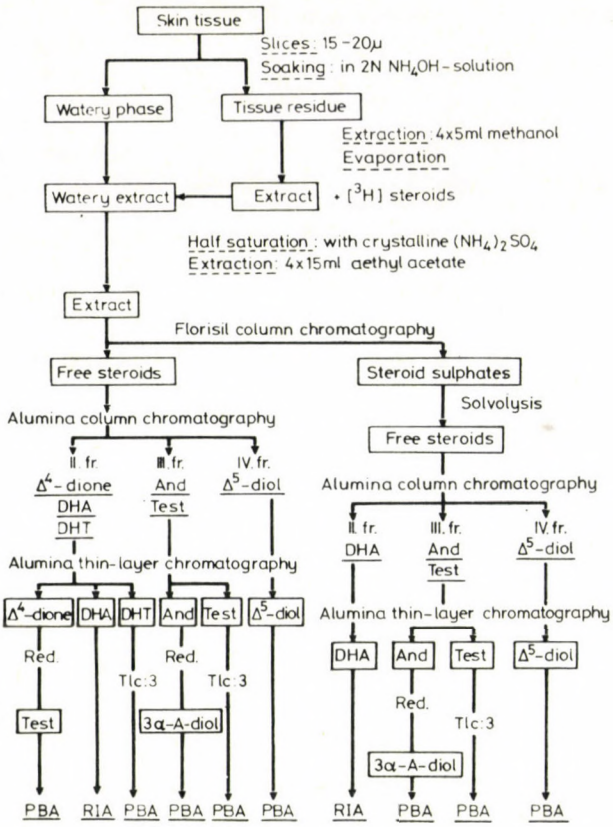

Fig. 1

A further 5 ml 2 N NH_4OH was added to the skin tissue residue, which was then triturated strongly with a glass rod. Centrifugation followed, and the supernatant (5 ml) was combined with the previous one (total 15 ml).

The skin tissue residue was next triturated with 4×5 ml methanol, and after centrifugation the methanol extracts were combined (20 ml) and then evaporated to dryness in vacuum at 30 °C.

Tritium-labelled variants of the steroids to be measured (with known radioactivities: 100,000–150,000 dpm) were then added to the dry residue, followed by 15 ml 2 N NH_4OH.

The combined extract was half-saturated by the addition of 6 g crystalline $(NH_4)_2SO_4$, and the mixture was shaken with 4×15 ml ethyl acetate. After centrifugation, the combined ethyl acetate extract (60 ml) was evaporated to dryness in vacuum at 30 °C. Water traces were removed by evaporation in vacuum after the addition of ethanol and benzene.

Separation of free and sulphate ester steroids

The crude extract was dissolved in 1 ml methanol, and the fractions of free steroids and sulphate esters were separated by chromatography on a 1.5 g florisil column suspended in 24 ml absolute benzene [16].

Isolation and determination of free androgens

The fraction of free androgens was chromatographed on a 4 g Al_2O_3 column and separated into fractions I, II, III and IV.

Fraction II contained the Δ^4-dione, DHA and DHT; fraction III, the And. and Test.; and fraction IV contained the Δ^5-diol.

Fraction II and the combined fractions III and IV were chromatographed in the TLC-"G" system, and the steroids with different polarities were separated (Figs 2 and 3).

The 17-ketosteroids (Δ^4-dione and And.) dissolved out of the layer were purified as Test. in the TLC-"G" system after $NaBH_4$ reduction, as was the 5α-androstane-3β, 17β-diol in the TLC-"C_2" system.

After being dissolved out of the layer, DHT and Test. (Figs 2 and 3) were further purified on a new thin-layer in the TLC-"3" system.

The adsorbent containing the steroids was scraped out of the thin-layer and collected in small tubes, and the mixture with the adsorbent was then transferred with 2×2 ml absolute benzene onto a 1 g florisil column suspended in benzene (7.5 cm high). The column was washed first with 10 ml absolute benzene, and then with 20 ml 0.5% methanolic benzene. The steroids were eluted from the column with 10 ml 10% methanolic benzene. The eluate (10 ml) was evaporated to dryness in vacuum at a temperature not higher than 30 °C. The dry residue was dissolved in 5 ml methanol, and radioactivity of the steroid was determined in 2×0.1 ml aliquots, so that a correction could be made for the loss arising during the purification procedure.

The individual steroids were determined in aliquots of the remaining 4.8 ml methanolic solution. DHA was determined by radioimmunoassay [13, 18], and the other steroids (Δ^5-diol, Test., DTH and 5α-ane-diol) by protein-binding assay [17]. The final result, corrected for the added radioactivity, was given in units of ng/g or nmol/kg, referred to wet skin tissue weight.

Isolation and determination of C_{19}-steroid sulphates

The sulphate ester fraction was evaporated to dryness and then solvolysed with 30 ml 1% perchloric acid in ether at 37 °C for 16–20 hours. The solution was extracted with 5 ml 5 N NaOH, and finally with 2×5 ml distilled water. The ether solution was evaporated to dryness, and tritium-labelled variants of And. and Test. of known radioactivity (100,000–150,000 dpm) were added. The tritiated DHA-S and Δ^5-diol-S were added to the extract during extraction. The extract of the solvolysed steroids was chromatographed on a 4 g Al_2O_3 column, and they were determined as free androgens as described above.

The quantities of free androgens measured (DHA, Δ^5-diol, And. and Test.) were converted to steroid sulphate esters, and the final result was given in units of ng/g or nmol/kg, referred to wet skin tissue weight.

Results are expressed as mean ± S.E.M.

Results

Of the free androgens in the abdominal skin of healthy males and females (Table I), DHA occurred in the greatest amount (165 ± 78, and 80 ± 28 nmol/kg, respectively). Whereas And. was present in almost the same concentration in the abdominal skin of males and females (48 ± 25, and 55 ± 19 nmol/kg/respectively) the concentration of Δ^4-dione was significantly higher in males (101 ± 36 nmol/kg) than in females (36 ± 8 nmol/kg). Δ^5-diol, Test and DHT were found in decreasing amounts in the abdominal skin, but all three androgen-active steroids were more abundant in males than in females.

Of the C_{19}-steroid sulphates (Table II), And.-S (132 ± 29 nmol/kg) was present in the highest concentration in the abdominal skin of females, whereas DHA-S had the highest concentration (2162 ± 406 nmol/kg) in that of males. Δ^5-diol-S and Test-S were found in lower concentrations in both males and females, but here too there was a difference between the sexes: the concentration of DHA-S and Δ^5-diol-S were significantly higher in the males than in the females.

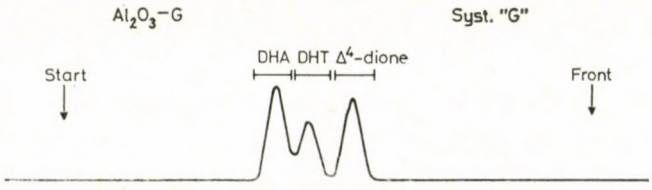

Fig. 2

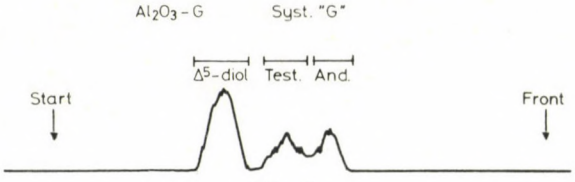

Fig. 3

Discussion

The values of the individual androgenic steroids determined in human abdominal skin represent the androgen concentration in the whole abdominal skin; this is due to a combination of the androgens reaching the skin via the blood stream and those formed in the skin. In the results no distinction was made between androgens in the cytosol, those in the cell nucleus, and those bound to receptors. The individual androgen concentrations in the whole abdominal skin may be influenced by the quantities found in the various appendages of the skin: the hair follicles, the apocrine sweat glands and the sebaceous glands.

Few data are available in the literature concerning the steroid content of skin [7]. Deslypere et al. [2, 3] determined the concentrations of three androgenic steroids (Test., DHT and 5α-A-3α, 17β-diol) in the skin of males. Evaluation of their results was hampered by the circumstance that they obtained the skin samples from corpses, and the metabolism occurring before examination in the samples may have caused considerable changes in the ratios of individual steroids. In spite of this, they demonstrated that the androgen concentration was highest in scrotal skin, lower in pubic skin, and even lower in the skin excised from the thigh. This means that the steroids can be compared only in skin excised from a given area of the body. In all cases, we carried out steroid analysis in skin from the abdomen, and thus the observed steroid concentrations reflect the in vivo hormonal state of abdominal skin.

If the steroid concentration measured in abdominal skin are compared with those of peripheral blood, for each of the free steroids a substantially

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Table I

Concentration of androgenic steroids in abdominal skin of normal women and men (ng/g and nmol/kg)

Steroids determined	Concentrations	No of cases	Normal women Limits	(28–46 yr) Mean ± S.E.M.	No of cases	Normal men Limits	(28–47 yr) Mean ± S.E.M.	P
DHA	nmol/kg	7	21.5–406.0	80.3 ± 28.15	5	43.0–465.0	165.2 ± 77.66	N. S.
	ng/g		6.2–117.0	23.1 ± 8.11		12.4–134.0	47.6 ± 22.40	
And	nmol/kg	6	5.5–111.1	54.7 ± 19.36	5	0.5–97.0	48.1 ± 24.95	N. S.
	ng/g		1.6–32.3	15.9 ± 5.63		0.16–28.3	14.0 ± 7.28	
Δ^4-dione	nmol/kg	8	12.9–74.3	35.7 ± 8.07	5	37.0–237.0	101.2 ± 35.55	$P < 0.05$ S.
	ng/g		3.7–21.3	10.24 ± 2.31		10.6–68.0	29.1 ± 10.19	
Δ^5-diol	nmol/kg	6	6.5–92.2	29.80 ± 13.66	5	8.0–55.0	34.6 ± 7.54	N. S.
	ng/g		1.9–26.8	8.70 ± 3.97		2.2–16.1	10.1 ± 2.23	
Test	nmol/kg	7	1.7–43.7	16.50 ± 6.06	5	17.0–65.0	34.8 ± 9.17	N. S.
	ng/g		0.7–12.6	4.80 ± 1.73		4.8–18.6	9.9 ± 2.63	
5α-DHT	nmol/kg	7	0–28.5	8.5 ± 4.03	5	0–48.0	20.6 ± 9.28	N. S.
	ng/g		0–8.3	2.5 ± 1.17		0–14.0	6.0 ± 2.69	

N. S. = not significant
S = significant

Table II

Concentration of C_{19}-steroid sulphates in abdominal skin of normal women and men (ng/g and nmol/kg)

Steroids determined	Concentrations	No of cases	Normal women Limits	(28–46 yr) Mean ± S.E.M.	No of cases	Normal men Limits	(28–47 yr) Mean ± S.E.M.	p
DHA-S	nmol/kg	6	7.0–186.4	81.95 ± 26.71	5	976–3065	2162±406	P < 0.001 S.
	ng/g		2.6–68.8	30.30 ± 9.86		360–1131	798±150	
And-S	nmol/kg	8	22.1–206.3	132.2 ± 29.06	5	31–364	204±64	N. S.
	ng/g		8.2–76.4	49.0 ± 10.76		11.5–134.8	75.5 ± 23.7	
Δ^5-diol-S	nmol/kg	6	15.9–62.4	42.1 ± 7.14	4	49–235	132.5 ± 41	P < 0.05 S.
	ng/g		5.9–23.1	15.6 ± 2.64		18.3–87.0	49.2 ± 15.2	
Test.-S	nmol/kg	7	4.3–25.7	14.2 ± 3.09	5	3.5–60.0	22.1 ± 10.0	N. S.
	ng/g		1.6–9.5	5.3 ± 1.14		1.3–22.0	8.1 ± 3.69	

N. S. = not significant
S. = significant

higher concentration was found in the skin than in serum. Thus, free androgens accumulate in the skin. The situation is different for the C_{19}-steroid sulphates, the blood level of which is several orders of magnitude higher than that of free steroids. Under normal conditions the values of these steroids were much lower in abdominal skin than in peripheral blood.

An increasing number of data support the assumption that the androgen-sensitivity of tissues may be characterised by the number of androgen-receptors, the latter varying in parallel with the concentration of androgens. Thus, the higher androgen concentration found in the hairy abdominal skin of males indicates a higher level of steroid-binding proteins in the skin. Confirmation of this will be a task of subsequent examinations.

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STEROIDS EXCRETED BY HUMAN SKIN II. C_{19}-STEROID SULPHATES IN HUMAN AXILLARY SWEAT

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Beside dehydroepiandrosterone sulphate and androsterone sulphate, 5-androstene-3β, 17β-diol-3-sulphate and testosterone sulphate were isolated and identified from the axillary sweat of sexually mature women and men. The quantities of these four C_{19}-steroid sulphates were determined individually in the sweat of eight healthy, sexually mature women and men: dehydroepiandrosterone sulphate was found in the highest amount, it was followed by 5-androstene-3β, 17β-diol-3-sulphate and androsterone sulphate. Testosterone sulphate occurred in the lowest quantity, its excretion amounting to about one-thousandth of that of dehydroepiandrosterone sulphate. Study of the C_{19}-steroid sulphates excreted by the apocrine sweat glands permitted an insight into the steroid metabolism in human skin, which presumably is, closely connected with the steroid hormones of peripheral blood.

Keywords: axillary sweat, C_{19}-steroid sulphates, human skin

Terms and abbreviations: Androstanedione (A-ane-dione): 5α-androstane-3,17-dione; Androstenedione (Δ^4-dione): 4-androstene-3,17-dione; Androsterone (And.): 3α-hydroxy-5α-androstan-17-one; Androstenediol (Δ^5-diol): 5-androstene-3β, 17β-diol; Dehydroepiandrosterone (DHA): 3β-hydroxy-5-androsten-17-one; Dihydrotestosterone (DHT): 17β-hydroxy-5α-adrostan-3-one; Testosterone (Test.): 17β-hydroxy-4-androsten-3-one; Dehydroepiandrosteron sulphate (DHA-S): 3β-sulphooxy-5-androsten-17-one; Androstenediol sulphate (Δ^5-diol-S): 5-androstene-3β, 17β-diol-3-sulphate; Androsterone sulphate (And.-S): 3α-sulphooxy-5α-androstan-17-one; Testosterone sulphate (Test.-S): 17β-sulphooxy-4-androsten-3-one

Introduction

Our investigations on the steroid hormone content of axillary hair demonstrated that the bulk of the C_{19}-steroids detected are to be found in the form of water-soluble sulphate esters [14]. Our experiments showed that these steroids can be extracted from the hair by soaking [8, 14], and it was therefore assumed that the hair takes up the steroid sulphates from sweat.

The quantity of C_{19}-steroids determined in axillary hair does not however, give reliable information on the amount of steroid sulphates excreted by the human skin or the sweat glands. An exact answer to this question can be given only by analysis of axillary sweat collected in a definite period of time.

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The present paper reports on quantitative examinations of the four most important C_{19}-steroid sulphates excreted in axillary sweat: dehydroepiandrosterone sulphate (DHA-S), androsterone sulphate (And.-S), 5-androstene-3β, 17β-diol--3-sulphate (Δ^5-diol-S) and testosterone sulphate (Test.-S).

Materials and methods

1. The chemicals and solutions employed were of analytical purity. The organic solvents were purified by prescribed procedures, and distilled in a fractionating column [8].

2. Radioactive steroids: [7-^{3}H(N)] DHA-S (S. A.: 24 Ci/mmol), [7-^{3}H(N)]Δ^5-diol-S (S. A.: 24 Ci/mmol) and [1,2-^{3}H(N)] And. (S. A.: 40 Ci/mmol) were products of New England Nuclear (USA), while [1β, 2β(n)-^{3}H] Test. (S. A.: 44.6 Ci/mmol) was obtained from the Radiochemical Centre (Amersham, England). These steroids were purified on Al_2O_3 column and on thin-layer [8].

3. Radioactivity was measured with a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3375) with correction of the quench effect. The efficiency of the instrument for ^{3}H in the case of free steroids was 45–48%, while in the case of steroid sulphates (where the scintillation medium contained also 1 ml methanol) it was 33–35%.

4. After thin-layer chromatography, localization of the spots of the radioactive steroids was performed with a Packard Radiochromatogram Scanner (Model 7201).

5. The free and sulphate ester steroids were separated on florisil column (Florisil 60/100 mesh; Floridin Co., USA) [12]. The free steroids of different polarities were separated on Al_2O_3 column with benzene containing an increasing ethanol concentration gradient [8]. The free steroids of similar polarity were separated on Al_2O_3-G (nach Stahl, Merck, GFR) thin-layers with the solvent system (n-hexane-ethyl acetate — absolute ethanol — glacial acetic acid 120 : 130 : 1 : 2 (v/v/v/v)) [8].

6. Chemical reactions of C_{19}-steroids

a. Reduction: the 17-ketone group can be reduced with a methanolic solution of $NaBH_4$ under appropriate experimental conditions to yield a hydroxy group [8].

b. Acetylation. When acetic anhydride is added to a solution of the C_{19}-steroid in pyridine, the hydroxy groups are acetylated [8].

c. The steroid acetates are hydrolysed in alkaline methanol, and the free steroids can be extracted with dichloromethane [8].

d. Oxidation. The hydroxy groups of the C_{19}-steroids can be converted to ketone groups by oxidation with chromic acid [8].

e. The C_{19}-steroid sulphates are solvolysed by perchloric acid [11].

7. Quantitative determination of the steroids. The amounts of DHA-S and And.-S present in sweat were determined by means of the Zimmerman and dinitrophenylhydrazine colour reactions [8, 11]. The amount of Δ^5-diol-S and Test.-S were measured by protein-binding assay. The serum of women in the third trimester of pregnancy was used as binding protein [13, 14].

8. Sweat collection. Gauze pads were used to collect the sweat for quantitative examinations. A 0.5 cm thick layer of cotton-wool was sewn between two layers of gauze measuring 10×15 cm. The resulting pads were tied onto the examined individual by means of six elastic tapes so that the arm remained free, but at the same time the armpits were completely covered (Fig. 1). Sweat was collected for 24 hours from both armpits, the axillary hair having first been shaved off and the surface of the skin cleaned with cotton-wool. The pads were then transferred to a glass-filter attached to a suction-bottle, and extracted with 3×15 ml methanol. The combined methanolic solution was evaporated to dryness in vacuum.

9. Individuals examined. These were healthy women and men, who were predominantly laboratory workers in our Department, and performed their normal daily activities at the time of sweat collection.

10. Brief description of determination of C_{19}-steroid sulphates in axillary sweat. The course of our procedure is outlined in Fig. 2. [^{3}H]DHA-S and [^{3}H]Δ^5-diol-S of known activities were added to the crude extract obtained by methanolic dissolution from the sweat collector, in order to be able to correct for the loss arising during the isolation procedure, and also to be able to localize the steroids to be determined during their chromatographic purification. The lipids were removed by defatting from the crude extract containing a large amount of contamination [8], and the fraction of steroid sulphates was separated by column chromatog-

raphy on florisil as described previously [8, 12]. Known quantities of [^{3}H] And. and [^{3}H] Test. were added to the extract of the free steroids obtained after solvolysis, and the material was chromatographed on Al_2O_3 column. Final isolation of the individual steroids was performed on Al_2O_3 thin-layer. The quantities of steroid sulphates determined in axillary sweat were expressed in units of nmol/24 h or pmol/24 h. Results are expressed as mean ± S.E.M.

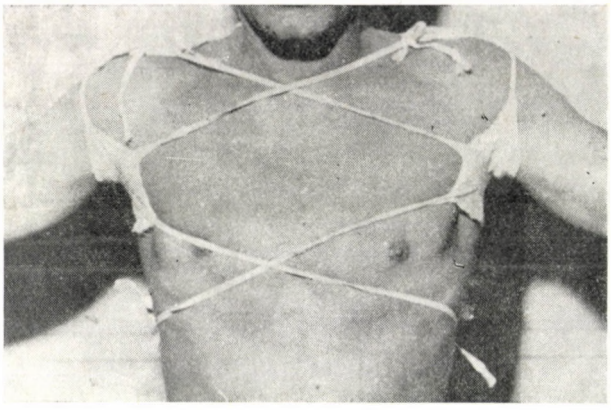

Fig. 1. Collection of axillary sweat

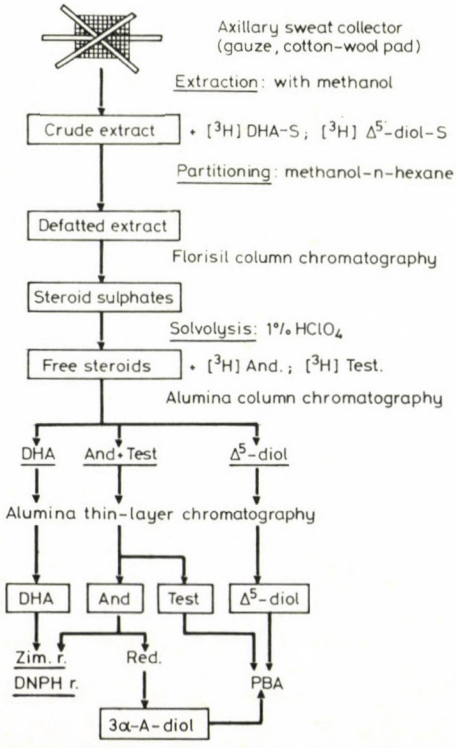

Fig. 2. Determination of C_{19}-steroid sulphates in axillary sweat: flow-sheet

Results

1. *Identification of Δ^5-diol-S and Test.-S*

Since DHA-S and And. -S were identified earlier in axillary sweat [8], we deal here only with the identification of Δ^5-diol-S and Test.-S.

After chromatographic separation, the quantity and radioactivity of the individual isolated steroids were determined on aliquots, and their specific activity (S. A.) was calculated. In the residual part Δ^5-diol and Test. were subjected to the chemical reactions shown in Tables I and II, respectively,

Table I

Identification of Δ^5-diol-S isolated from axillary sweat following solvolysis

Subject	[7-^{3}H]Δ^5-diol-S added to sweat extract (S.A. = 24 Ci/mmol)	Chemical reactions	Derivatives	TLC systems	S.A. (dpm/μg)	Amount of Δ^5-diol-S in sweat (nmol/24 h)
J. T.	535.752 dpm	—	Δ^5-diol	G	8850	165
25 yr		Acetylation	Δ^5-diol-diAc	$1a_0$	—	
male		Hydrolysis	Δ^5-diol	G	7764	186
B. T.	470.322 dpm	—	Δ^5-diol	G	6030	211
31 yr		Acetylation	Δ^5-diol-diAc	$1a_0$	—	
female		Hydrolysis	Δ^5-diol	G	6391	200
T. I.	507.230 dpm	—	Δ^5-diol	G	3800	359
43 yr		Acetylation	Δ^5-diol-diAc	$1a_0$	—	
male		Hydrolysis	Δ^5-diol	G	4180	327

S. A. = Specific Activity
T L C s y s t e m s:
G = Al_2O_3-G, n-hexane-ethyl acetate-absolute ethanol-glacial acetic acid (120 : 130 : 1 : 2, v/v/v/v)
$1a_0$ = silica gel G, c-hexane-ethyl acetate (80 : 20, v/v)

and then, after the individual steps, were chromatographed in the appropriate thin-layer system. The chromatograms of the different steroids, and the scannogram of the labelled steroids running together with them, indicated that these two steroids isolated from sweat were identical with Δ^5-diol and Test.

Besides the qualitative proof, quantitative determination and S. A. measurement were repeatedly carried out after the chemical reactions; these results are also given in Tables I and II.

The tabulated date show clearly that the S. A. values obtained after chromatographic running of the derivatives produced in the chemical reac-

tions, and the quantities of sweat steroid calculated from these, did not differ essentially from the results measured initially. This unambiguously demonstrated the identity of Δ^5-diol-S and Test.-S, and showed that our procedure for their isolation and determination was exact and reliable.

Table II

Identification of Test.-S isolated from axillary sweat following solvolysis

Subject	[1β,2β-^{3}H] Test. added to sweat extract (S.A. = 44.6 Ci/mmol	Chemical reactions	Derivatives	S.A. (dpm/ng)	Amount of Test.-S in sweat (pmol/24 h)
Zs. J.	185.078 dpm	—	Test.	358	1403
21 yr		Oxidation	Δ^4-dione	—	
female		Reduction	Test.	354	1417
T. I.	191.549 dpm	—	Test.	991	526
43 yr		Oxidation	Δ^4-dione	—	
male		Reduction	Test.	1004	518
T. I.	191.549 dpm	—	Test.	393	1319
44 yr		Oxidation	Δ^4-dione	—	
male		Reduction	Test.	420	1237

S. A. = Specific Activity

2. *Amount of C_{19}-steroid sulphates excreted in 24 h*

The quantities of C_{19}-steroid sulphates excreted in the axillary sweat of eight sexually mature women aged 17–37 years and eight sexually mature men aged 17–44 years are listed in Table III. As observed in a study of the C_{19}-steroid sulphates of axillary hair [14], DHA-S was present in the highest amount in both sexes (women: 795 ± 233 nmol/24 h; men 1063 ± 189 nmol/24 h. The amount of And.-S in axillary sweat was substantially lower (women: 51 ± 16 nmol/24 h; men: 66 ± 13 nmol/24 h). A difference could be seen between the mean values for the women (74 ± 24 nmol/24 h) and the men (152 ± ± 37 nmol/24 h) as regards the excretion of Δ^5-diol-S, though the individual scatter was wide in both groups. Of the four C_{19}-steroid sulphates examined, Test.-S was excreted in lowest amount in axillary sweat (women: 815 ± ± 367 pmol/24 h; men: 2055 ± 939 pmol/24 h).

The data in Table III clearly revealed the large individual fluctuations for both women and men. It could also be observed that certain individuals (B. K. a 21-year-old woman, and J. T., a 26-year-old man) intensively excreted all four steroid sulphates, whereas lower than average amounts of all four steroid sulphates were found in the sweat of others (M. V. and T. I., 20 and 37-year-old women).

3. *Ratios of C_{19}-steroid sulphates*

The constancy of composition of the steroids excreted in sweat was demonstrated in an experimental series in which the quantities and ratios of the sweat steroids excreted daily by two men were determined on several occasions. The data in Table IV show that with the exception of Test.-S, the amounts

Table III

Amounts of C_{19}-steroid sulphates in axillary sweat of healthy women and men

Subject	Age (years)	DHA-S nmol/24 h	And.-S nmol/24 h	Δ^5-diol-S nmol/24 h	Test.-S pmol/24 h
1. K. E. female	17	505	38	46	288
2. T. M. female	17	1688	148	70	934
3. T. M. female	18	152	13	16	280
4. V. K. female	19	901	65	111	—
5. M. V. female	20	125	19	11	624
6. B. K. female	21	1769	76	111	442
7. B. T. female	31	982	35	208	2942
8. T. I. female	37	242	16	19	195
Limits:	17—37	125—1769	13—148	11—208	195—2942
mean ± S.E.M.		795±233	51±16	74±24	815±367
9. B. T. male	17	564	24	46	—
10. Sz. F. male	23	573	67	76	6339
11. J. L. male	25	1202	46	159	—
12. J. T. male	26	2217	132	386	1514
13. D. I. male	27	787	65	86	393
14. L. J. male	35	841	54	189	2915
15. T. J. male	42	1243	35	135	597
16. T. L. male	44	1077	105	138	573
Limits:	17—44	564±2217	24—132	46—386	393—6339
mean ± S.E.M.		1063±189	66±13	152±37	2055±939

Table IV

Amounts and ratios of C_{19}-steroid sulphates in axillary sweat collected from healthy subjects at various times

Subject	Date of sampling	DHA-S nmol/24 h	And.-S nmol/24 h	Δ^5-diol-S nmol/24 h	Test.-S pmol/24 h	DHA-S / And.-S	Δ^5-diol-S / And.-S
J. T.	24 Jan.	2217	132	386	1514	16.8	2.9
26 yr	26 Jan.	2252	135	486	1319	16.7	3.6
male	31 Jan.	2114	127	435	3981	16.6	3.4
T. I.	16 Nov.	1077	105	138	573	10.2	1.3
43 yr	18 Nov	2014	219	359	1189	9.2	1.6
male	22 Nov.	3297	321	418	4285	10.3	1.3
	1 Dec.	2250	208	281	1319	10.8	1.3

of C_{19}-steroid sulphates in the axillary sweat of J. T., a 26-year-old man, varied scarcely at different times. For the second individual, T. I., a 43-year-old man, however, the quantities of steroids found in sweat differed substantially in the repeated sweat collections. The ratios of the excreted steroids DHA-S/And.-S and Δ^5-diol-S/And.-S were seen to vary with the individual, but to be fairly constant and characteristic of each individual (DHA-S/And.-S = 16.6–16.8 and 9.2–10.8, and Δ^5-diol-S/And.-S = 2.9–3.6 and 1.3–1.6), independently of the amounts of the individual steroid sulphates and the time of sampling.

Discussion

When our steroid studies were extended from axillary hair [14] to axillary sweat, we could demonstrate that, besides DHA-S and And.-S, a considerable amount of Δ^5-diol-S too is excreted in human axillary sweat. It is known that Δ^5-diol occurs in peripheral blood not only as monosulphate, but also as disulphate [10]. It should be noted that with our procedure we determined in sweat Δ^5-diol-3-S, i.e. the monosulphate form.

In earlier in vitro incubation examination we showed that Δ^5-diol-S was present among the metabolites when Δ^5-diol was incubated with skin slices, i.e. the Δ^5-3β-hydroxysteroid-sulphokinase in human skin is able to form a sulphate ester not only from DHA, but also from Δ^5-diol [5, 6]. Thus, part of the Δ^5-diol-S excreted in sweat may be Δ^5-diol-S from peripheral blood, while part may originate from the sulphate metabolite formed in the skin.

Little is known of the physiological significance of Test.-S; its plasma level was very low compared to those of the other C_{19}-steroid sulphates [4]. We have demonstrated the identity of Test.-S found in sweat, by means of chemical reactions and chromatographic methods. Its quantity was only one-thousandth of that of DHA-S in sweat. During in vitro incubation with skin slices, Test.-S formation has never been observed.

Two types of sweat gland can be distinguished in human skin: the small eccrine and the larger apocrine glands. We earlier showed that there was no measurable amount of steroid in sweat collected in the abdominal area [8]. Since the apocrine sweat glands occur in greatest number in the armpit, and only eccrine glands are found in the abdominal skin, it has been concluded that the apocrine glands were responsible for the excretion of steroid esters, the eccrine sweat glands playing no role in this [2, 3]. This assumption was supported by our experiments which indicated that there was no detectable amount of steroid sulphate in the axillary sweat of girls and boys before puberty, when the apocrine glands are not yet functioning.

Δ^{16}-steroids (5α-androst-16-en-3α-ol, 5α-androst-16-en-3-one) with properties of pheromones have recently been found in axillary sweat [1, 2, 9] but their quantities are far lower than those of the steroid esters we have measured.

The amounts of the examined C_{19}-steroid sulphates varied between very wide limits in the different individuals, and these differences can be explained only in part by the individual plasma steroid level. It is assumed that the extent of exretion depends primarily on the number and activity of apocrine sweat glands of the individual. This was shown by the sweat steroid determinations on different days: the amount of C_{19}-steroid sulphates excreted by the apocrine glands varied in time, depending on the state of emotional stress of the individual [1], while the composition of excreted steroids (as expressed by the ratios DHA-S./And.-S and Δ^5-diol-S/And.-S) was almost constant at different points of time. This varied from individual to individual, but was characteristic of a given individual, presumably being determined by the individual plasma steroid composition.

The quantity of C_{19}-steroid sulphates excreted in sweat is so extensive that, together with the excretion of steroids in urine, this is one of the most important routes of steroid elimination. Its importance does not, however, lie in the quantity; study of the sweat steroids gives insight into the steroid metabolism of human skin, which is closely connected with the steroids of peripheral blood.

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Gynecology

QUANTITATIVE CHANGES IN STEROID AND PEPTIDE HORMONES IN THE MATERNAL-FETOPLACENTAL SYSTEM BETWEEN THE 28th—40th WEEKS OF PREGNANCY

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The role of steroid and peptide hormones in the maintenance of pregnancy and in the onset of labour was investigated in the maternal-fetoplacental system between the 28th–40th weeks of pregnancy. The data of 151 pregnant women and 108 newborns was processed, and a total of 2385 samples was studied by RIA.

Results showed that between the 28th–40th week there is an increase in the prolactin level of the umbilical arterial and venous blood with a parallel increase in the cortisol level of umbilical arterial blood and a fall in the progesterone level of umbilical arterial and venous blood which between the 36th and 40th week results in a rise of the oestradiol/progesterone quotient. At the same time the oestradiol concentration rises in the maternal circulation and in case of premature labour the oestradiol/progesterone quotient attains values twice as high as those found in normal pregnancies. The alteration of the hormonal environment expedites the predominance of the mechanisms stimulating the activity of the myometrium. The hormone values in the umbilical artery and vein characteristically differ from those of the maternal serum. Thus, it may be assumed that the fetus, though being in close communication with the maternal organism, is capable of independently maintaining its hormonal environment. The results indicate that the fetus or more accurately the fetoplacental unit plays a significant part in the maintenance of pregnancy and in the initiation of labour than it has been assumed previously.

Keywords: maternal-foetoplacental system, initiation of labour, hormonal levels, feto-hormonal independence

Introduction

It is known that approximately 70% of the deaths within the first week of life occur in preterm infants. The rate of preterm birth in Hungary has risen from 1950 to the seventies from 5% to 10–12%. Owing to the high morbidity and mortality rate of preterm infants, investigation of the causes inducing preterm delivery and prevention of these are essential tasks of obstetric medicine, tasks requiring a closer insight into the normal and abnormal processes of pregnancy.

Studies in the last two decades have resulted in significant discoveries. We have the organ perfusion studies of Diczfalussy [2] to thank for the recog-

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niton of fetoplacental hormonal units. Liggins [3], Nathanielsz [5], Thornburn [6] and associates studied the hormonal changes in the course of gestation and at the onset of parturition by means of a catheter inserted into the mother animal and its fetuses, and found a sharp prenatal rise in the plasma cortisol level of sheep fetuses, parallel with a decline of the progesterone level. Subsequently, the oestradiol and prostaglandin concentrations rose in the maternal serum.

Data relating to humans and their interpretation are contradictory. Fetal and maternal values have scarcely been considered together, and observation of preterm births is particularly rare in this respect. It is mainly at term that the hormonal values in maternal and umbilical arterial and venous cord blood have been studied. The maternal values, however, provide indirect information about the fetoplacental secretory activity. On the other hand it is precisely the hormonal activity of the fetus which studies of mixed cord blood fail to disclose. For these reasons samples serving for the present study were taken from the maternal cubital vein and separately from the fetal umbilical artery and umbilical vein. This has allowed us to study in the maternal-fetoplacental unit, the hormonal changes forming part of the mechanisms responsible for the onset of labour. The studies included the stage of preterm labour as well. The study was concerned with the following questions.

1. What changes occur in the hormonal levels of the sera of the maternal vein, the fetal umbilical vein and artery between the 28th–40th week of pregnancy?

2. What differences are there in the serum hormonal levels in normal gravidae and in premature parturients between the 28th–36th week of pregnancy?

Material and methods

Data of 151 gravidae parturients and 108 newborns were processed (Table I).

Table I

The clinical groups under study

Group I: Premature delivery (28—36 week):		
	74 parturients	
		74 premature newborns
Group II: Full-term delivery (week 40):		
	34 parturients	
		34 newborns
Group III: Normal gravidae (28—36 week) acting as controls:		
	43 gravidae	
Gravidae and parturients, total:	151	
		108 newborns

Of the parturients 74 gave birth to newborns weighing 1001 to 2500 g between the 28th and 36th weeks of pregnancy.

The results obtained in premature parturition were compared with those of 34 full-term parturients. The serum hormone values of these parturients were compared with those found in the same week of normal pregnancy in 43 gravidae. Premature parturients were not given steroid prophylaxis, parturition did not begin with preterm rupture of membranes and intrauterine retardation was not perceived in any of the newborn. The material was collected partly at the Mother and Infant Protection Research Centre of the Soviet Ministry of Health in Moscow, partly at the Second Department of Obstetrics and Gynaecology, Semmelweis University Medical School in Budapest.

Samples were taken from the maternal cubital vein, the fetal umbilical vein and the fetal umbilical artery separately, immediately after delivery, before expulsion of the placenta. We wished to examine, as far as possibilities permitted, those hormone-producing organs within the maternal-fetoplacental-unit which, on the evidence of published data, are essential factors of this system. Information on pituitary function was provided by the prolactin level, on placental secretory activity by the progesterone and HPL levels, on adrenocortical activity by DHAS and cortisol and on the fetoplacental-unit by oestradiol and oestriol.

The serum hormone concentration was determined by RIA in a total of 2385 samples (Table II). Progesterone, oestradiol, oestriol, cortisol and prolactin concentration was deter-

Table II

Number of hormone assays

Hormones	Maternal vein	Umbilical vein	Umbilical artery
Progesterone	148	106	91
DHAS	149	106	100
Oestradiol	148	107	94
Oestriol	148	106	94
Cortisol	148	107	94
Prolactin	144	106	92
HPL	141	91	65
Total:		2385 assays	

mined in the hormone laboratories of the Mother and Infant Protection Research Centre of the Soviet Ministry of Health in Moscow using the reagents of the "Steranti" firm according to the WHO protocol (London). DHAS levels were measured at the Second Department of Obstetrics and Gynaecology, Semmelweis University Medical School, Budapest, following the Buster and Abraham method [1], and using the isotopes of the Amersham firm and the Abraham's antiserum. HPL concentration was determined at the Frederic Joliot-Curie Institute of Radiobiology and Radiohygiene, Budapest, following the Mohari–Kocsár method [4], using the reagents of the U.S. Biochemical Corp. In the determination of hormone concentration the interassay variance coefficient and the intraassay variance coefficient values were as follows: Progesterone 7.8%, 12.5%; oestradiol 4.6%, 9.1%; oestriol 7.5%, 8.4%; cortisol 5.7%, 10.2%; DHAS 7.2%, 14.5%; HPL 9.8%, 9%, prolactin 6.8%, 11.2%.

Through mathematical processing of the data the mean values and S.E.M. of the individual hormones were calculated and the results were subjected to analysis of variance in order to establish whether the hormonal values were affected by the gestational age. On these grounds the period between the 28th and 40th weeks was divided into three groups: 28–32th, 33–36th and 40th weeks. Then each analysis was carried out separately in the three groups. The serum hormone concentration in the maternal vein, the umbilical artery and the umbilical vein were compared, also by analysis of variance. The two sample *t*-test was used for comparison of the values found in normal gravidae versus premature parturients. All variants were correlated to each other. For these calculations a computer type IBM 30/31 belonging to the Hungarian Academy of Sciences was used with the aid of the "BMPD Statistical Software 1981 edition".

Results

1. *Serum steroid and peptide hormone levels in maternal venous, umbilical venous and umbilical arterial blood between the 28th–40th week of pregnancy.*

Figure 1 shows the *progesterone* concentrations. While in maternal blood a significant rise occurred between the 28th and 40th week, the progesterone concentration of the cord blood rose only between the 28th and 36th weeks and thereafter it fell significantly. The highest progesterone concentration was

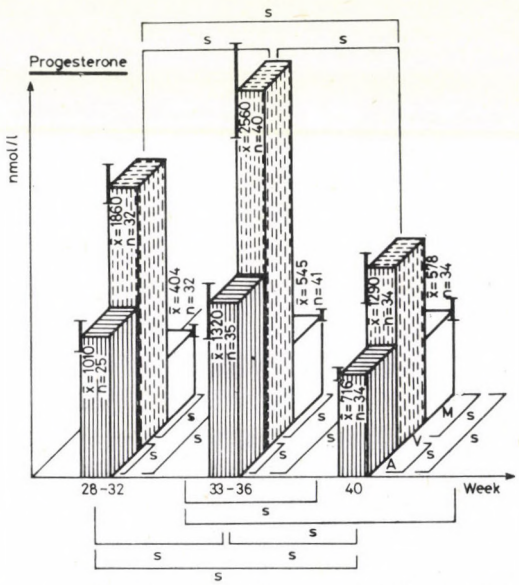

Fig. 1. Progesterone concentration in serum of maternal vein, umbilical vein and umbilical artery between the 28th and 40th weeks[1]

found in the umbilical vein but that of the umbilical artery was also significantly higher than the progesterone level in maternal blood ($P < 0.01$). Whereas changes in the hormone concentration in the umbilical arterial or venous blood failed to correlate with those in maternal blood, a close correlation was found between the values of the unbilical vein and artery ($r = 0.7783$, $P < 0.01$).

The *DHAS levels* (Fig. 2) of maternal blood showed a rising tendency between the 28th and 36th weeks, and a significant fall between the 36th and

[1] On the three-dimensional diagram. The hormone concentrations have been shifted on the perpendicular axis.
Columns representing the serum hormone concentrations:
Empty columns: maternal blood "M"
Interrupted lines: umbilical vein "V"
Hatched lines: umbilical artery "A"
n = number of cases. *In each figure:* $\bar{x}$ = mean; *scatter:* ± S.E.M.; s = significance

40th week ($P < 0.01$). At the same time the values of the umbilical vein and the umbilical artery tended to increase. DHAS concentration in the umbilical artery was significantly higher than in maternal blood ($P < 0.01$). While the maternal DHAS concentration correlated only loosely with the values for the umbilical vessels (M-V: $r = 0.2951$, $P < 0.01$) the correlation between those of the umbilical artery and vein was very close ($r = 0.8432$, $P < 0.01$).

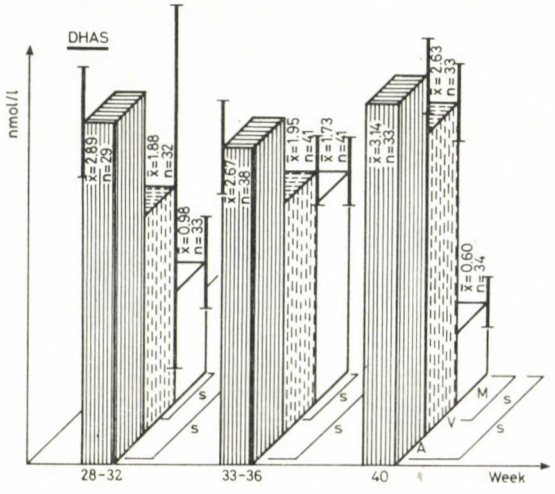

Fig. 2. DHAS concentration in serum of maternal vein, umbilical vein and umbilical artery between the 28th and 40th week

The oestradiol concentration (Fig. 3) of maternal blood rose significantly between the 28th and 40th weeks ($P < 0.01$), whereas that of the umbilical artery and the umbilical vein increased only between the 28th and 36th week, to fall significantly thereafter ($P < 0.01$). Its concentration in maternal serum was significantly higher than either in the umbilical vein or in the umbilical artery ($P < 0.01$). While the oestradiol level in maternal blood correlated only loosely with the level in umbilical blood, there was a close correlation between the concentration in the umbilical venous and arterial blood (M-V: $r = 0.2672$, 0.01 M-A: $r = 0.6462$, $P < 0.01$).

The oestradiol-progesterone quotient (Fig. 4) was studied next. A rise of the quotient between the 28th and 40th weeks was confined to the umbilical vein whereas in the umbilical artery it rose between the 28th and 36th weeks but it ($P < 0.01$) failed to rise in maternal blood.

The oestriol concentration (Fig. 5) in the maternal blood rose significantly ($P < 0.05$) between both periods of 28th—32th and 33rd—36th weeks. The values of the umbilical vein and artery also rose significantly between the 28th and 36th week ($P < 0.05$) but fell to half the original figures by

the 40th week ($P < 0.01$). The highest serum oestriol levels were found in the umbilical vein ($P < 0.01$) but the values measured in the umbilical artery between the 28th and 36th weeks were also higher than those of the maternal blood ($P < 0.01$). While the oestriol concentration in maternal blood correlated only loosely with those in the fetal vessels (M-V: $r = 0.3677$, $P < 0.01$) there

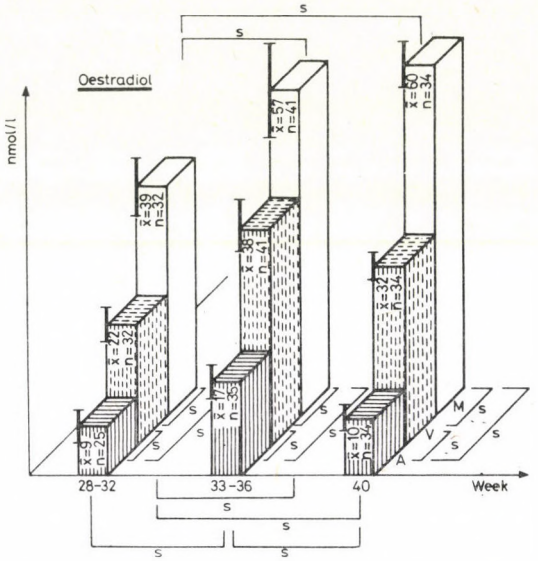

Fig. 3. Oestradiol concentration in serum of maternal vein, umbilical vein and umbilical artery between the 28th and 40th week

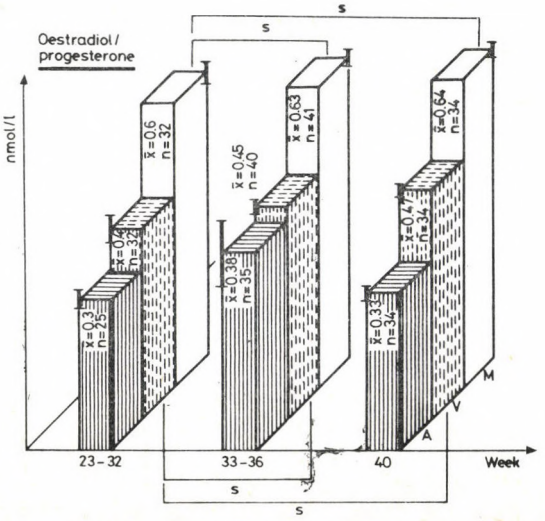

Fig. 4. Oestradiol/progesterone quotient in serum of maternal vein, umbilical vein and umbilical artery between the 28th and 40th week

was a close correlation between the levels observed in the umbilical vein and artery ($r = 0.7073$, $P < 0.01$).

As regards the *cortisol level* (Fig. 6) its significant increase between the 28th and 40th weeks was confined to the umbilical artery ($P < 0.01$). Cortisol concentration was significantly higher in the maternal blood than in the umbi-

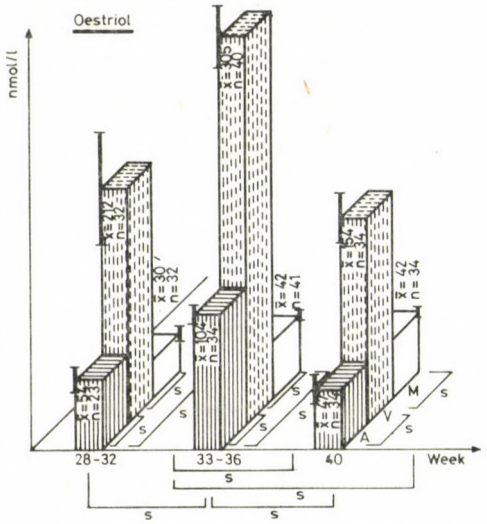

Fig. 5. Oestriol concentration in serum of maternal vein, umbilical vein and umbilical artery between the 28th and 40th week.

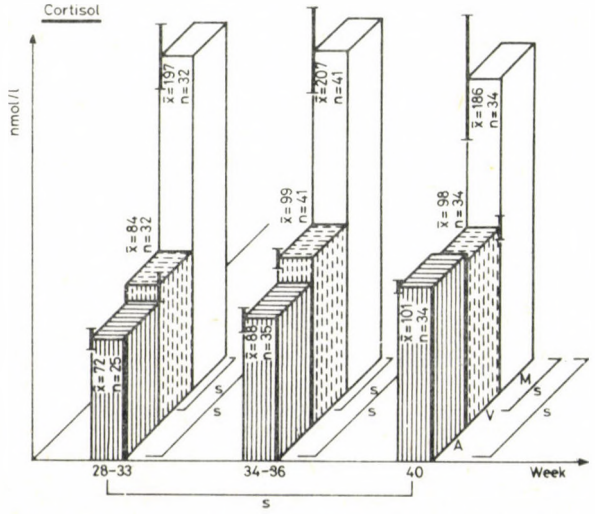

Fig. 6. Cortisol concentration in serum of maternal vein, umbilical vein and umbilical artery between the 28th and 40th week

lical vein and the umbilical artery ($P < 0.01$). A close correlation was observed between the maternal venous and the umbilical arterial serum cortisol levels (M-V: $r = 0.7165$, $P < 0.01$; M-A: $r = 0.5952$, $P < 0.01$; V-A: $r = 0.9126$, $P < 0.01$).

As to the *prolactin level* (Fig. 7) between the 28th and 40th week only in the umbilical vein and the umbilical artery was a significant rise ($P < 0.01$),

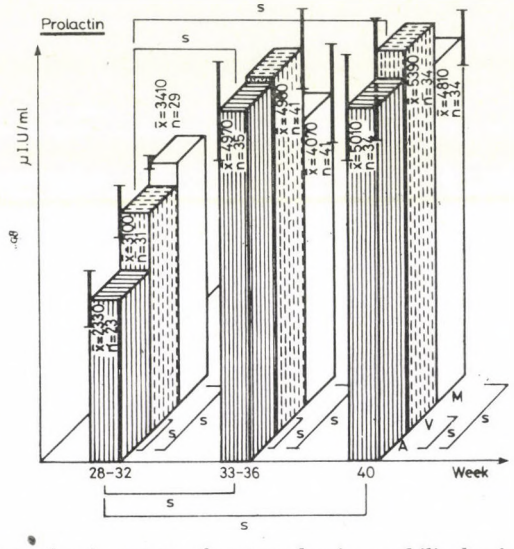

Fig. 7. Prolactin concentration in serum of maternal vein, umbilical vein and umbilical artery between the 28th and 40th week

found but not in the maternal blood. While between the 28th and 36th weeks the prolactin level of maternal blood was higher than that of the umbilical artery and vein, ($P < 0.01$) between the 33rd and 36th weeks and during the 40th week. The blood of the umbilical artery and vein showed significantly higher values ($P < 0.01$) than the maternal blood. The serum prolactin level of the maternal vein did not correlate with that in the umbilical artery and only loosely with that in the umbilical vein ($r = 0.2152$, $P < 0.05$). There was a slight correlation between the cortisol levels in the umbilical vein and artery ($r = 0.4991$, $P < 0.01$).

The *HPL level* (Fig. 8) tended to rise between the 28th and 40th week in the maternal blood, but fell significantly ($P < 0.01$) in the umbilical vein, and artery ($P < 0.01$). The serum HPL concentration of maternal blood failed to correlate with that in the umbilical vein and artery, but the values in the umbilical vessels showed a close correlation $P < 0.01$ $r = 0.63996$.

2. *Comparison of the serum hormone values of preterm parturients and those of normal gravidae*

During the 28th–32nd weeks and the 33rd–36th weeks of pregnancy the preterm parturients had significantly higher values for DHAS, oestriol, the oestradiol/progesterone quotient (Figs 9–10) and cortisol than did normal gravidae. On the other hand in normal gravidae the HPL values were significantly higher than in preterm parturients ($P < 0.01$).

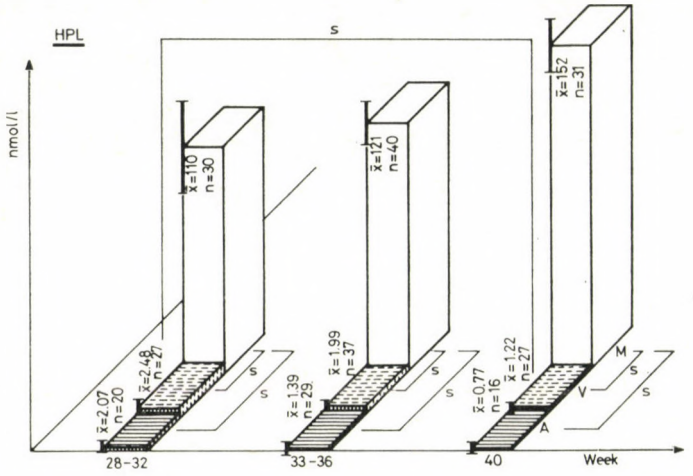

Fig. 8. HPL concentration in serum of maternal vein, umbilical vein and umbilical artery, between the 28th and 40th week

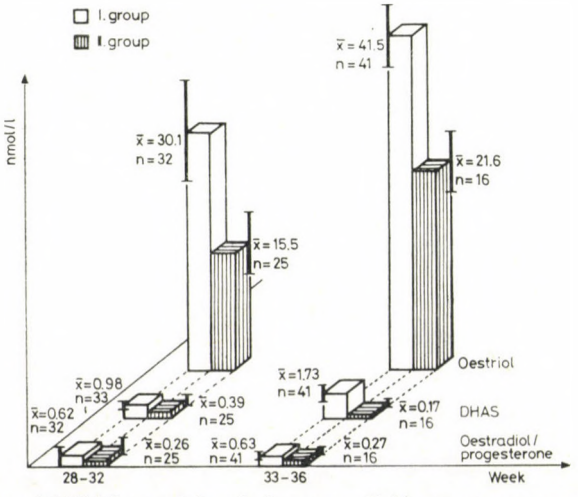

Fig. 9. Concentration of DHAS oestriol and the oestradiol/progesterone quotient in serum of normal gravidae and of preterm parturients between the 28th and 36th week. 1st group: premature labour; 2nd group: normal pregnancy

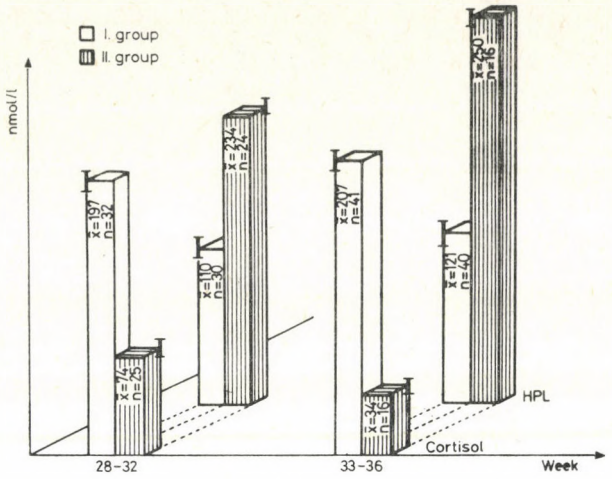

Fig. 10. Concentration of cortisol and HPL in serum of normal gravidae and of premature parturients between the 28th and 36th week. 1st group: premature labour; 2nd group: normal pregnancy

Discussion

Although the hormonal changes accompanying the onset of labour in humans are less spectacular than those found in laboratory animals, the processes that take place in the maternal-fetoplacental unit are nonetheless typical. Between the 28th and 40th weeks of pregnancy we found an increase in the prolactin level in the umbilical artery and vein together with a high cortisol level in the umbilical artery. Parallel with the rise of cortisol concentration, the concentration of progesterone in the umbilical vessels declined between the 36th and 40th weeks, thus the oestradiol/progesterone quotient rose during the 28–40th week in the umbilical vein and during the 28th–36th week in the umbilical artery. At the same time the oestradiol level increased in the maternal circulation, and the oestradiol/progesterone quotient attained in the preterm parturients twice the value found in normal pregnancy.

With the increase of the cortisol level in the maternal-fetoplacental unit the oestradiol/progesterone balance was shifted in the direction of oestradiol. The activating mechanism of the myometrium thus gained preponderance as a result of changes in the hormonal environment.

The hormone values found in the umbilical artery and vein differed characteristically from those in maternal serum. The changes in the concentration of progesterone, prolactin and HPL in umbilical cord blood failed to correlate with those in the maternal blood, in other words the concentration of these hormones changed in the fetal circulation independently of the maternal organism.

The sera of the maternal vein and of the fetal vessels displayed typical differences in the quantitative proportion of the hormone levels under study. While in the 8th to the 40th week the oestradiol, cortisol and HPL and in the 28th to 36th weeks the prolactin concentration level was significantly higher in the maternal sera than in that of the fetus, in the 36th to 40th week those of progesterone, DHAS, oestriol and prolactin were significantly higher in the umbilical artery and vein. This typical proportion of the hormone values seems to suggest that the fetal organism, in spite of its close communication with the maternal organism, is capable of maintaining its hormonal environment independently.

On the basis of the present results the fetoplacental unit seems to play a more independent, more active part in the maintenance of pregnancy and in the initiation of labour than it has hitherto been assumed.

Acknowledgements

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COMPARATIVE STUDY OF INTRAVENOUS ANAESTHETIC TECHNIQUES ADMINISTERED DURING SHORT-TERM GYNECOLOGICAL OPERATIONS

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The effects of nine intravenous anaesthetic techniques on serum cortisol level and circulatory parameters were studied in healthy patients undergoing termination of pregnancy.

Considering the increased pre-operation values and those of the pregnant control group as well as diurnal variations, it was found that the serum cortisol value changed during and after the procedure depending on the type of anaesthesia used. This might mean that the optimal type of anaesthesia for interruption could be designed by measuring the serum cortisol level, assuming that this parameter was one of the indicators of stress. The rating the different types of anaesthetics, the circulatory parameters were also considered.

According to these criteria the best results were observed using methohexital in combination with fentanyl or diazepam.

Keywords: serum cortisol, pregnancy termination, intravenous anaesthesia, stress

Introduction

It is well known that operative procedures cause pain accompanied by an increase of adrenal function [2, 5, 8, 14]. Many authors have studied the stress-effect during different types of anaesthesia and surgical intervention [1, 2, 11, 15, 17]. The plasma cortisol concentration, was found to increase during surgical stimulation and to remain higher than the basal value for a variable time after the operation [4, 7]. The serum cortisol level is an accepted index for evaluating the degree of surgical stress [2, 11, 14, 15].

Few data are available about the effect on the adrenal response during minor surgery of short-term intravenous anaesthetics and their combinations with analgetics or sedatives. In the present study the differences in this respect of various short-term anaesthetic techniques used for pregnancy termination have been analysed.

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Material and methods

Healthy patients (n = 16) who underwent termination of pregnancy in the first trimester were studied after informed consent. Mean age was 29.0 ± 7.0 (mean ± S.D.) years, mean body-weight 63.3 ± 12.7 kg and the stage of pregnancy was between 5 and 14 weeks. All patients were classified in the ASA I group. The operation were carried out between 11 a.m. and 1 p.m. and lasted 9–15 min.

Nine different groups were formed on the basis of the administered intravenous anaesthetics analgetics and benzodiazepine (Table I).

Table I

Patients groups according to type and dosage of drugs administered for anaesthesia

Groups	n	Fentanyl * μg/kg	Diazepam mg/kg	Propanidid mg/kg	Thiobutabarbital mg/kg	Methohexital mg/kg
Propanidid	11	—	—	18.6 ± 7.3*	—	—
Fentanyl + Propanidid	10	1.65 ± 0.73	—	14.6 ± 4.1	—	—
Diazepam + Propanidid	14	—	0.16 ± 0.04	12.2 ± 3.9	—	—
Thiobutabarbital	14	—	—	—	14.4 ± 4.4	—
Fentanyl + Thiobutabarbital	14	1.21 ± 0.33	—	—	12.4 ± 2.9	—
Diazepam + Thiobutabarbital	11	—	0.10 ± 0.04	—	11.2 ± 2.4	—
Methohexital	10	—	—	—	—	2.88 ± 0.69
Fentanyl + Methohexital	12	1.29 ± 0.43	—	—	—	2.58 ± 0.61
Diazepam + Methohexital	10	—	0.18 ± 0.02	—	—	2.24 ± 0.70

Mean ± S. D.

Premedication was not applied before interruption. The anaesthetic analgetic fentanyl and benzodiazepine (diazepam) were administered fractionally until the patients had been slightly sedated and one of the intravenous anaesthetics propanidid, thiobutabarbital and methohexital was injected until disappearance of the eyelid reflex. Spontaneous respiration was maintained in all cases. During anaesthesia, 250–300 ml Ringer-lactate solution was infused.

For estimation of the serum cortisol level, four blood samples were taken in all cases from the antecubital vein before the introduction of anaesthesia, during surgery, after awakening (i.e. when the patient was able to respond adequately to questions), and at 6 hours p.m.

In the control group (n = 10) blood-samples were taken at 6, 12 and 6 hours for the purpose of determining diurnal variations of cortisol in the first trimester of pregnancy. The blood samples were centrifuged and the separated serum was stored at −30° until analysed.

Serum cortisol was measured by radioimmunoassay [18]. The antigen was cortisol-21-hemisuccinate-BSA. The antibody titer was: 1 : 21000, its specifity: cortisol 100%, cortisone 25%, corticosterone 2.2%, 11-deoxycortisol 40%, progesterone 0.5%, 17-alpha-hydroxy-progesterone 10%, 11-alpha-hydroxy-progesterone 1%, the normal range being 160–660 nmol/l. The accuracy of this method: inter-assay (variation coefficient): ±14.2%, intra-assay (v.c.): ±10.8%. Blood pressure and pulse rate were measured at the time of blood sampling.

The relative changes of cortisol, blood pressure and pulse rate compared to the pre-operation values were determined all drug-combinations. In view of the marked individual differences, the diurnal variations as well as the physiological distribution of cortisol, relative changes were studied. In the course of statistical analysis cortisol levels measured at different time were compared to the pre-operation values.

Statistical analysis was performed using the Wilcoxon test for estimating differences of the various drug combinations, blood sampling times and cortisol levels. The drug-combinations were evaluated according to the parameters obtained and the rank of the different types of anaesthesia was established using regression analysis. The results were expressed as mean ± S.D.

Results

Before examination of the different types of anaeshesia, it was established that the diurnal variation of serum cortisol in the first trimester of pregnancy was similar to that of non-pregnant women, but at a higher concentration range (at 6 a.m. 582 ± 229 nmol/l, at 12 noon 351 ± 148 nmol/l and at 6 p.m. 266 ± 112 nmol/l), (Fig. 1). Therefore in this study the cortisol values in pregnant women were considered as controls.

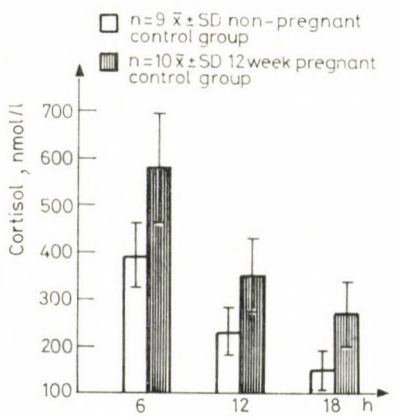

Fig. 1. Diurnal variation of serum cortisol level in pregnant and non-pregnant women

Serum cortisol values around 12 noon are considerably higher than in the control group (603.3 ± 67 vs. 351 ± 148 nmol/l), a fact which could be explained by the excitement before interruption, as no premedication was applied.

The relative changes of the serum cortisol level during interruption and after anaesthesia are shown in Fig. 2, where mean values are given.

The predicted variations of cortisol values before, during and after interruption were compared using the Wilcoxon test. Significant differences in the cortisol level between the pre-operation value and the value expected after surgery were observed only in the diazepam + propanidid and diazepam + + thiobutabarbital groups ($P < 0.01$). During further differentiation the cor-

tisol values decreased during interruption in the fentanyl-methohexital and diazepam + methohexital groups (Fig. 3). The values were unchanged or increased in approximately the same degree in the remaining 5 groups.

In those groups in which propanidid was the basic agent (propanidid, fentanyl + propanidid, diazepam + propanidid), the cortisol level increased

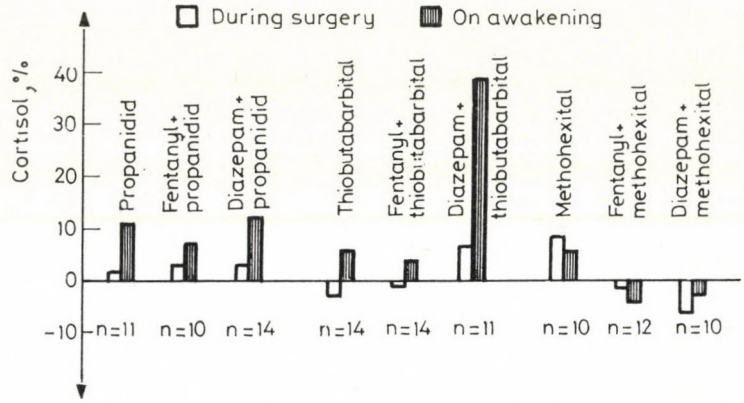

Fig. 2. Serum cortisol level during interruption and on awakening as compared to pre-operation values

but the increase was significant ($P < 0.01$) only with the diazepam + propanidid combination.

During anaesthesia when the basic anaesthetic agent was thiobutabarbital, the cortisol level showed a decrease during interruption (—2.6%) and an increase on awakening (+5.8%) when barbiturate was administered as a single drug. No change was observed in the cortisol values during anaesthesia compared to the pre-operation value when the barbiturate was combined with fentanyl (—0.1%). However, on awakening the cortisol level increased in this group too, but less (+3.7%) than with the use of thiobutabarbital alone (+5.8%). The increase was also significant when the barbiturate was combined with diazepam (+7.0% and 38.6%), respectively.

The best results were measured in the groups which received methohexital, in spite of the fact that during anaesthesia with methohexital alone the cortisol level increased (+8.9%), whereas on awakening it showed a decreasing tendency (+5.9%). If methohexital was combined with fentanyl or diazepam, the serum cortisol level decreased (fentanyl + methohexital: —1.2%, diazepam + methohexital: —6.7%), and the decrease lasted when subsequently only fentanyl was applied (—3.7%).

Comparing the 6 p.m. values the cortisol level in the fentanyl + methohexital and diazepam + methohexital groups did not differ significantly from the control. In the groups of fentanyl + thiobutabarbital, diazepam + thiobuta-

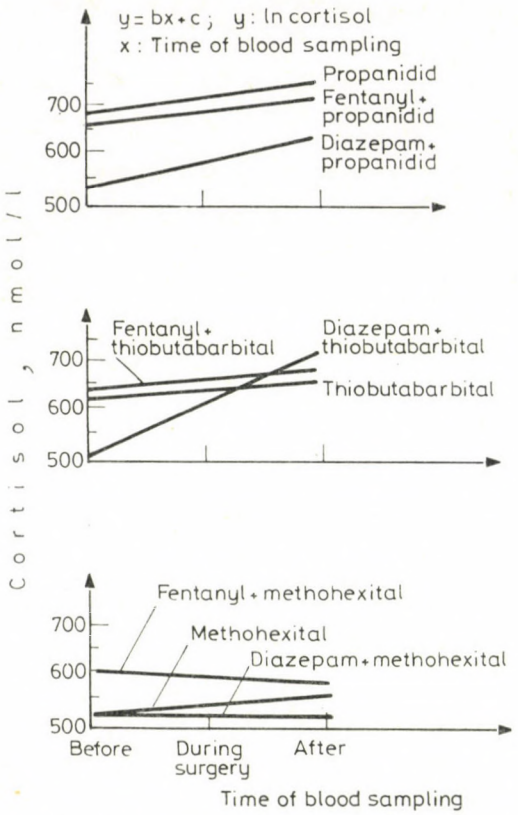

Fig. 3. Cortisol level during interruption during administration of different anaesthetic techniques

barbital and methohexital the cortisol levels differed significantly ($P < 0.05$). When propanidid, fentanyl + propanidid, diazepam + propanidid and thiobutabarbital were used, the differences were even more significant ($P < 0.01$) compared to the pregnant control group's 6 p.m. cortisol values (Fig. 4).

Blood pressure decreased moderately during interruption and in the post-operative period in all groups. The decrease ranged from 5 to 15% but statistically the difference was not significant (Fig. 5). The pulse rate increased by 15% in the fentanyl + propanidid, diazepam + thiobutabarbital and methohexital groups (Fig. 6), but not during operative interventions. At 6 p.m. the pulse-rates decreased except in the fentanyl + propanidid and diazepam + thiobutabarbital groups.

Evaluation of the drug-combinations was performed by calculating the regression co-efficients. From the drug-combinations the one which had a smaller regression co-efficient was considered most effective. The best results

were observed with fentanyl + methohexital and diazepam + methohexital (Table II). In these cases the regression co-efficients were negative.

The doses of short-acting intravenous anaesthetics could be decreased if they were combined with analgetics or sedatives.

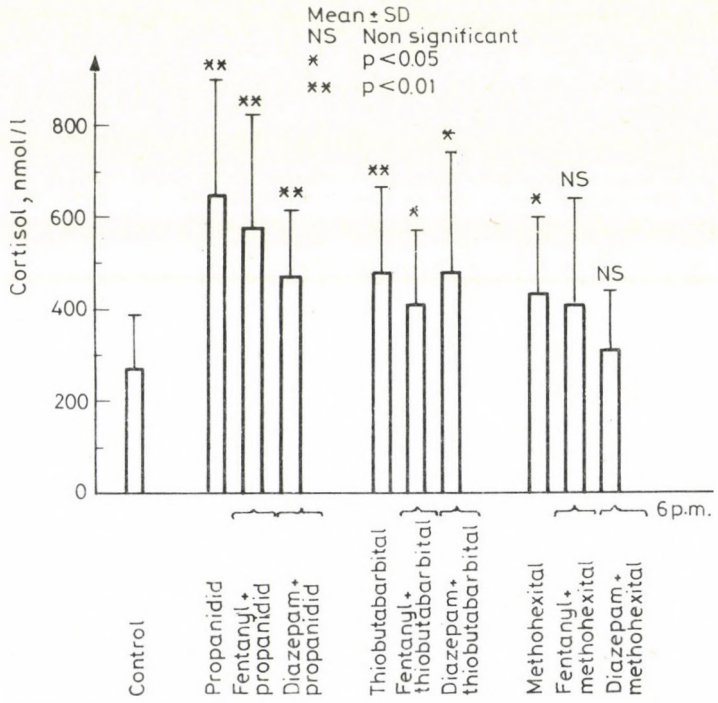

Fig. 4. Serum cortisol values at 6 p.m. compared to values at 6 p.m. in pregnant control group

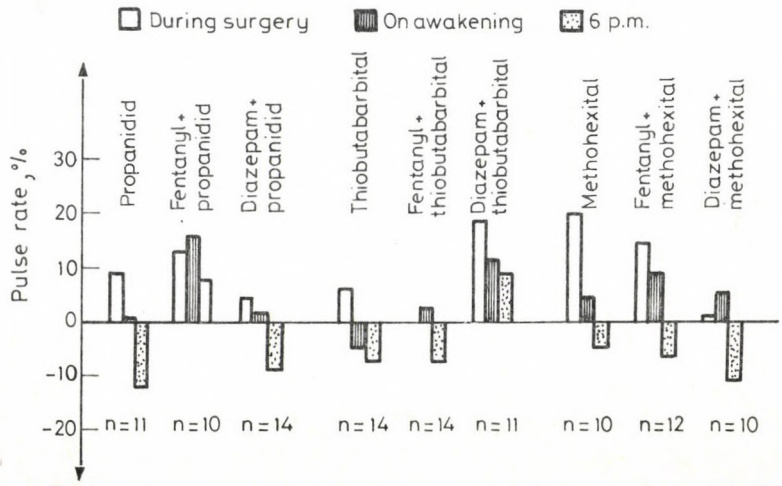

Fig. 5. Relative changes in blood pressure as compared to pre-operative values

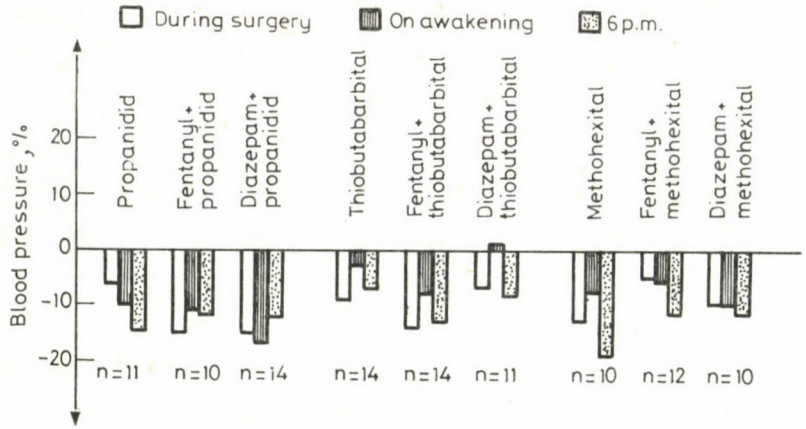

Fig. 6. Relative changes in pulse rate as compared to pre-operative values

Table II

Rank of different anaesthesia types related to the changes of serum cortisol levels

Rank of groups	Narcotic-analgetic drugs	Regression coefficients	Significance between the regression coefficients
1.	Fentanyl + Methohexital	−0.0254	NS; $P < 0.05$
2.	Diazepam + Methohexital	−0.0060	$P < 0.05$
3.	Thiobutabarbital	0.0266	
4.	Methohexital	0.0271	
5.	Fentanyl + Thiobutabarbital	0.0302	
6.	Fentanyl + Propanidid	0.0449	
7.	Propanidid	0.0519	$P < 0.05$
8.	Diazepam + Propanidid	0.0839	
9.	Diazepam + Thiobutabarbital	0.1659	

Equation of the analysed relation: $y = bx + c$

where:

y = ln cortisol

x = time of blood sampling (before, during and at the end of surgery)

b, c = parameters of linear regression relation

NS: non-significant

Discussion

In recent years for characterizing and quantitating the different stress-situations, the serum catecholamine and cortisol levels were measured [1, 2, 5, 8, 17].

Plasma cortisol has been found to rise during surgery and its changes to be similar as those of the blood sugar level [6, 15, 16] A parallel rise in ACTH level was found and presumably this was responsible for the increase of cortisol [10], but the plasma ACTH concentration was far higher than that required to produce a maximum adrenocortical response [13]. In addition, the normal pituitary-adrenocortical feedback mechanism is no longer effective as the concentration of both hormones increases simultaneously. ThisA CTH administration during surgery produces no further increase in plasma cortisol, and corticosteroid administration in the postoperative period does not abolish the ACTH-cortisol response [13].

Apart from increased production, a rise in plasma cortisol could also result from decreased breakdown. A reduction in hepatic blood flow during anaesthesia has been shown and the liver is responsible for the metabolism of hydrocortisone by conjugation [12]. The elimination half-life of cortisol in the body takes approximately 2 hours, so that this factor would be of little importance during the first hour of surgery. It seems therefore more likely that the production of cortisol is related to the degree of surgical stress. A considerable rise of the plasma cortisol level has been shown during major surgery and a smaller rise during minor operations [11]. The extent of the rise thus varies with the type of surgery and anaesthesia.

Anxiety and fear which increase cortisol secretion and blood cortisol values before an operation have been shown to be influenced by premedication and the sleep pattern of the previous night [9]. In the period after operation factors such as infection, prolonged bed rest, hypoxaemia and even alterations in the usual day-night physiological cycles all contribute to the changes.

The neuroendorcrine response to surgery may be altered in different ways. The purpose is the reduction or abolition of the endocrine and metabolic changes that result from surgical stimulation [3].

The neuroendocrine response to trauma appears to have evolved to assist survival in a primitive environment by providing appropriate substrates to maintain the function of vital organs. However, in modern anaesthetic and surgical practice where severe physiological disturbances are prevented or rapidly treated, and suitable drugs and agents made readily available, the benefits of the stress response are no longer apparent. The aim in the future must be the safe prevention of the adverse hormonal and metabolic changes induced by surgical stress.

The nine different drug-combinations administered in this study had a similar clinical benefit, but could be ranked in respect of anaesthesia according to the serum cortisol value. On the basis of this, estimation of the cortisol level related to operations seems to be an appropriate method for characterizing the different anaesthetic techniques and to suggest the optimum drug-combination for short-term anaesthesia. The measurement of serum cortisol levels could provide further details when new anaesthetics are introduced into clinical anaesthesia.

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Immunology

THE EFFECT OF SERA OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS ON ARTIFICIAL IMMUNE COMPLEXES

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The solubilization of artificial immune complexes mediated by complements has been well-known since 1975. It is known that the process is bound to the integrity of the alternative complement pathway. The phenomenon of solubilization can be used in the investigation of the function of the complement system. We have studied the solubilization of artificial complexes containing BSA and ^{125}I labelled anti-BSA on the effect of sera of healthy subjects and those of patients suffering from SLE. We have observed that the solubilization capacity of SLE sera is significantly lower than that of healthy persons. The decrease is the most distinct at the time of the activity of the disease.

Keywords: complement solubilization of immune complexes, systemic lupus erythematosus

Abbreviations: BSA — bovine serum albumin; SLE — systemic lupus erythematosus; CIC — circulating immune complexes; PEG — polyethylene glycol

Introduction

The solubilization of artificially produced immune complexes resulting on the effect of fresh serum was described by Miller and Nussenzweig [7]. In recent years the mechanism of the phenomenon has become clear even in its details. As an interaction of the immune complexes and the serum factors, a molecule group of C-3b-convertase activity joins with the large-sized complexes containing antigens and antibodies, ensuring first the incorporation of C-3b into the complex and then of its effect breaking up of the lattice structure into smaller units [7, 9, 10]. The complexes originating in this way differ from the starting complexes in their biological features [6]. The phenomenon is bound to the integrity of the complement alternative pathway, it postulates the presence of B, P, D factors as well as a required quantity of C-3, it is inhibited by anti-B serum while the presence of anti-C-2 serum or the absence of late com-

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plement components do not influence development of the reaction [9, 10]. The phenomenon of solubilization presented an opportunity for elaborating a new quantitative method in the investigation of the function of the complement system [1, 2, 7].

When the fresh serum of healthy subjects is given to artificially produced radiolabelled immune complexes, they will show a standard level with small fluctuations at different times, under constant experimental conditions, without distinction as to sex and age [8]. A decrease of the solubilization capacity of the serum was, however, observed in the case of patients suffering from autoimmune or immunogenetic disease [1, 8]. Although no parallelism was found between the increase of circulating immune complex level and the decrease of the solubilization capacity, a close correlation was observed between the decreased C-3 complement level and the reduced solubilization capacity [1].

In our experiments the solubilization capacity of the serum samples of patients with SLE has been investigated. Our aim was to compare the results obtained in the active and inactive stages of the disease within the patient group as well as the values in SLE nephropathy and those not due to renal involvement. In some patients the alterations of the solubilization capacity were followed for several months.

Materials and methods

Serum samples. The serum samples of 25 healthy subjects and 65 patients, considered to suffer from SLE on the basis of international criteria were studied. 45 out of the 65 serum samples were obtained in the inactive stage of the disease and 20 cases in the active stage. 17 samples originated from patients with nephropathy and 48 from patients without renal involvement. The blood was allowed to clot at room temperature for one hour and then at +4 °C for 2 hours. Thereafter the serum was separated by centrifugation. In the present experiments the sera were used immediately after separation, 1 ml of each sample was inactivated at 56 °C for 30 minutes and used as negative control.

BSA-anti-BSA complex labelled with ^{125}I. Labelling the BSA (Calbiochem) with ^{125}I was performed by the chloramine-T method [5]. Cleaned rabbit IgG produced against BSA (Miles Lab.) was used for preparing the complex. At weighing the labelled BSA with anti-BSA an approximately fourfold antibody excess was applied. The reaction mixture was incubated at 37 °C for 12 hours and the precipitate was separated by centrifugation at 1600 *g* for 30 minutes. The precipitate was washed six times in PBS buffer (physiological NaCl solution buffered with phosphate, containing 0.15 mmol/l Ca^{++} and Mg^{++} ions) centrifuged at 1600 *g* for 30 minutes and dispersed by repeated sucking through a thin cannule. The final concentration of the immune complex was presented in such a way that the standard sample produced from mixed sera of healthy subjects should solubilize 20 μl of the solution to 70%. At that time there was approximately 2–3 μg/20 μl protein in the solution containing IC.

Solubilization test. 400 μl of the 1 : 2 dilution of serum was measured to 20 μl IC solution in duplicate. Dilution of serum was performed with PBS containing Ca^{++} and Mg^{++} ions. The mixture was incubated at 37 °C for 2 hours under gentle stirring, then the reaction was stopped by adding 400 μl cold buffer. Segregation of the not solubilized precipitate and the supernatant was performed by centrifugation in a cooled centrifuge at 1600 g for 30 minutes. After segregation of the supernatant and precipitation the activity of each was measured. For determining the degree of spontaneous solubilization, the IC solution was incubated with PBS instead of serum. Calculation of the rate of solubilization was performed by the formula

$$\text{percent solubilization} = \frac{\text{activity of supernatant}}{\text{activity of supernatant and precipitate}} \times 100$$

Thereafter the value obtained was compared to a standard sample prepared from a 1 : 2 dilution of the mixed sera of healthy persons, considering the standard to be 100%. The value of spontaneous solubilization has been previously substracted from the values obtained. Simultaneously with the measuring of samples, the solubilization capacity of heat inactivated parallel sera was also determined; this value has never exceeded 10%, similarly to the spontaneous solubilization observed beside the buffer.

Anti-DNA levels and composition of CIC in sera were measured by micro-ELISA method from sera and 3.5% PEG precipitate of the sera, as described earlier [4].

Statistical analysis of the results was performed with Student's two-sample *t*-test. Results were expressed as mean ± S.D.

Results

In Table I the mean solubilization capacity of 25 serum samples from healthy persons and that of 65 serum samples with SLE are shown. The solubilization capacity of the serum of SLE patients compared to the standard was significantly lower than that of healthy person ($P < 0.001$). The values measured in the sera of patients were divided into groups accordings to whether at the moment of blood sampling disease was in an active or inactive stage. It was found that the mean solubilization capacity of the patients showing symptoms of activity was significantly lower than of those in the inactive stage ($P < 0.001$), although the mean value at the time of inactivity fell short of the mean of healthy persons. Results are illustrated in Table II.

Table I

Solubilization capacity of serum (mean ± S.D.)

Sera of healthy persons N: 25	97.72 ± 9.33%	$P < 0.001$
Sera of SLE patients N: 65	50.91 ± 13.92%	

N = number of patients

Table II

Solubilization capacity of SLE serum (mean ± S.D.)

SLE inactive N: 45	69.42 ± 14.95%	$P < 0.001$
SLE active N: 20	32.40 ± 12.90%	

N = number of patients

In Table III the results were divided into groups from the point of view of renal involvement. The mean solubilization capacity of serum from 17 SLE nephropathic patients was significantly weaker than that of SLE patients without any renal involvement ($P < 0.001$).

In Table IV the values of nephropathic patients were divided into groups according to the active or inactive stage of the disease at the time of blood sampling. In the active stage the mean solubilization capacity was significantly lower than that of nephropathic SLE patients in the inactive stage of the disease.

Table III

Solubilization capacity of serum of nephropathic SLE patients and those without any renal involvement (mean ± S.D.)

SLE with nephropathy N: 17	39.35 ± 21.56%	$P < 0.001$
SLE without nephropathy N: 48	64.56 ± 18.80%	

N = number of patients

Table IV

Solubilization capacity of serum of nephropathic SLE patients in the active and inactive stages of the disease (mean ± S.D.)

SLE active with nephropathy N: 10	24.52 ± 11.90%	$P < 0.001$
SLE inactive with nephropathy N: 7	60.57 ± 11.58%	

N = number of patients

In Table V, anti-DNA levels, immunoglobulins and C3 contents of CIC measure with ELISA are illustrated in the sera of active and inactive groups of patients and controls. We could find no correlation between solubilization capacity and anti-DNA titre and immunoglobulin contents of complexes. In the active stage the mean C3 content was significantly decreased ($P < 0.05$).

In Table VI data of the follow-up of 3 patients are shown.

Table V

Comparison of anti-DNA levels, components of PEG precipitates and solubilization capacity of sera (mean ± S.D.)

Patients	Anti-DNA level	3.5% PEG precipitates of sera		Solubilization capacity
		Immunoglobulin contents	C3 contents	
SLE active N: 20	37.27 ± 7.86	176.71 ± 24.62	0.93 ± 0.63	32.40 ± 12.90
SLE inactive N: 45	31.02 ± 16.46	157.02 ± 20.10	1.51 ± 1.18	69.42 ± 14.95
Healthy persons N: 25	19.5 ± 5.42	10.54 ± 1.62	n. m.	97.72 ± 9.33

Anti-DNA, immunoglobulin and C3 contents: μg/ml serum
n. m. = not measured
N = number of patients

Table VI

Solubilization capacity of sera of SLE patients at follow-up

Solubilization capacity	Activity	Patients
		1. Sz. I. (with nephropathy)
89	inactive	1982.09.01.
17	active	12.08.
17	active	12.14.
46	inactive	1983.02.16.
		2. N. J. (with nephropathy)
26	inactive	1982.10.13.
23	active	1983.02.02.
25	active	02.14.
48	inactive	05.25.
		3. N. E. (without nephropathy)
43	inactive	1983.01.02.
35	active	02.02.
31	active	02.23.
46	inactive	05.25.

Discussion

Relying upon data in the literature, we have worked out a method that is suitable for the investigation of complement mediated immune complex solubilization by a great number of serum samples. In accordance with other authors, on the basis of numerous preliminary experiments the method was found to be suitable for standardization and reproduction. The results of the method showed that the ability of sera of SLE patients to solubilize immune complexes considerably decreased as compared to that of healthy persons. The degree of decrease was most distinct in the active stage of the disease, 32.4% of that of healthy controls. The mean solubilization capacity of patients with SLE nephropathy was also considerably lower than the capacity of those without any renal involvement. The low value of the mean was however due to the low values of patients showing symptoms of activity.

From our follow-up investigations the data of 3 patients' are indicated. Although up to now we had only a small number of cases in addition to those where we succeeded to follow-up an essential alteration of the symptoms, it was observed that the decrease of the solubilization capacity precedes the clinical symptoms of activity and after the remission it reaches the starting level gradually, during several weeks. Among the data we have not indicated the values of the five serum, samples where there was an acute bacterial infection in the patients at the time of blood sampling. The values obtained at that time reached the level characteristic of healthy persons. The phenomenon has been observed by other authors as well [8] but its cause has never been clarified.

The decrease of in vitro immune complex solubilization capacity in SLE and other diseases of immune pathogenesis shows a correlation first of all with the low serum C3 level. One might suppose the presence of inhibitory materials or complement consumption as a cause of the decrease [8] Consumption of complement factors in these diseases may be the consequence of in vivo interaction of immune complexes being produced in great quantity.

C3 contents were significantly decreased in active stage CIC: Decreased C3 contents and reduced solubilization capacity indicated the presence of incompletely solubilized immune complexes in the circulation of SLE patients as it has been reported by Baatrup et al. [3].

In spite of the close correlation between the decrease of the C3 complement level and of the solubilization capacity, we must emphasize that the phenomenon of solubilization is the result of a compound mechanism dependent not only upon the C3 level but also on other factors and that is why one cannot demonstrate a complete parallelism in all, the cases. The aim of further investigations will be clarify how the measurement of the solubilization capacity of serum could be employed for following-up the clinical state, the activity and inactivity, and possibly also the effect of therapy.

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SERUM IgE LEVEL IN SYSTEMIC LUPUS ERYTHEMATOSUS

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The serum total-IgE level has been studied in systemic lupus erythematosus (SLE), in order to obtain data concerning the significance of IgE antibodies in the appearance of SLE symptoms. In most patients the serum IgE content was significantly higher in the active stage of the disease than during remission. The findings indicated a specific statistical distribution due to the multiple factors influencing IgE production and so an augmented IgE level cannot be regarded as a reliable feature of SLE activation.

Keywords: IgE, systemic lupus erythematosus, skin, allergy

Abbreviations: CDLE — chronic discoid lupus erythematosus; IgE — immunoglobulin E; SLE — systemic lupus erythematosus

Introduction

An important feature of systemic lupus erythematosus (SLE), one of the representative autoimmune diseases, is the increase of polyclonal immunoglobulins produced as a result of immunoregulatory disorder. In their 26 SLE patients, Rebhun et al. [15] found a correlation between clinical activity of the disease and the serum IgE content.

Mészáros et al. [13], too, have shown that, when compared with normal IgG, IgA and IgM concentrations, the IgE level was very high in CDLE patients.

An important concept concerning the regulation of IgE production was that of Marsh et al. [12], who postulated the role of double genetic regulation in the allergen-specific and non-specific IgE production.

By studying the serum total-IgE level in 95 SLE patients, we searched for an answer to the following questions. a) Is the increased immunoglobulin production observed in SLE valid for the IgE class? b) does the serum IgE content show an upward trend in the clinically active stage of SLE? c) is the quantitative development of the IgE level in SLE similar to that of other immunoglobulins? d) can the appearance of certain SLE skin symptoms be brought into connection with the serum IgE concentration? e) how does the

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IgE level of the patients develop during follow-up investigations? f) can any change of the IgE level be observed in SLE patients in connection with allergic manifestations?

From the results we have tried to conclude to the possible role of IgE antibody in the appearance of SLE symptoms and the course of the disease.

Materials and methods

A study was performed on 95 patients with SLE and 25 control subjects. SLE was diagnosed on the basis of the 1982 revised ARA criteria [18]. The active and inactive stages of the disease were defined on basis of the clinical signs and the laboratory parameters. In the period of the first examination 54 SLE patients were in remission and 41 in the active stage. The examination was later repeated in 46 patients on one or more occasions (follow-up examinations). Spontaneous (atopic) allergy and drug allergy were diagnosed basis of the history as well as clinical observations (the allergen-specific IgE level has not been measured).

The classification of SLE skin symptoms was performed on the basis of histological and immunohistochemical processing of skin biopsy materials in addition to evaluation of the clinical evidence.

The control group was selected from persons of corresponding age and sex, not suffering from autoimmune, allergic, inflammatory or tumourous disease.

Quantitative determination of the serum total-IgE level was performed by the method of Ceska and Lundkvist [2] by means of paper-disk radioimmunosorbent test (IgE PRIST, Phadebas, Pharmacia, Sweden) and of the serum immunoglobulins (IgG, IgA, IgM) by Mancini's radial immunodiffusion [10].

On account of the well-known wide distribution and the so-called lognormal distribution of the IgE level, logarithmic transformation of the values was also performed and geometric means were calculated [7]. We accepted the IgE level as decidedly increased if the value was above the geometric mean + 2 gSD (= 295 kU/l) of control sera [9].

Statistical analysis was performed by the U-test, Student's t-test and the χ^2-test.

Results

The distribution of the serum IgE levels observed in our material is illustrated in Fig. 1 and the results of measurements are summarized in Table I. Taking the arithmetic means as the basis, the IgE level of the SLE patients proved to be significantly higher than the average IgE level of the control subjects. The mean IgE level measured in the active period of the disease was significantly higher than that of patients in remission. The average IgE concentration of the patients in remission approximately equalled the mean of the healthy controls. The examination was repeated in 17 patients a later period of the disease. In this group, during the active period of the disease the mean IgE level was insignificantly higher than the value in the inactive stage and significantly higher as compared to the control value (Table I). Considering the wide scattering of the serum IgE level in the healthy adult population (7) and the lognormal distribution of the values different from the normal statistical distribution [7, 17], their logarithmic transformation was performed and then the difference between the geometric means proved to be significant only for the patients in the clinically active stage (Table I).

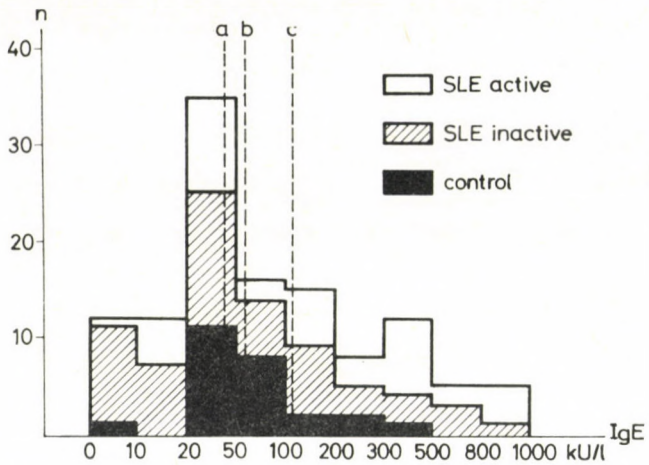

Fig. 1. Distribution of serum total-IgE levels in healthy and SLE population (n = 120) (a, b, c: geometric means; a = SLE inactive, b = control, c = SLE active)

Table I

Serum total-IgE concentration in SLE

Diagnosis	n		Arithmetic mean ± SD (kU/l)	Geometric mean ± gSE (kU/l)
SLE	Total	95	177.6 ± 243.8 $p < 0.01$ total to the control	61.8 ± 1.18 NS
*(independent examinations)	Inactive	54	119.5 ± 194.1	41.0 ± 1.23
	Active	41	254.2 ± 290.5 $p < 0.001$ active to the control $p < 0.01$ active to the inactive	105.9 ± 1.26 $p < 0.02$ active to the control $p < 0.01$ active to the inactive
SLE	Inactive	17	168.3 ± 208.8	74.6 ± 1.42
**(parallel examinations)	Active	17	231.0 ± 257.7 $p < 0.01$ active to the control	94.9 ± 1.47
Control		25	78.2 ± 80.5	53.9 ± 1.18

* the examination was performed in different patients

** the examination was performed in the same patients in the active and inactive periods of the disease

In SLE patients with a high (>295 kU/l) IgE level, the concentration of other immunoglobulins was also elevated as compared to that of patients with a low IgE level (Table II). The difference, however, was not significant. Figure 2 illustrates the results obtained by correlating three different SLE skin symptoms and the IgE level. In the group of patients with fixed erythema more displayed a high IgE level than in the group without any skin symptoms. In spite of the expectations among the patients with urticaria few had an IgE level above 295 kU/l. The differences between the geometric means were not significant statistically.

Follow-up examinations were performed in 46 patients. In half of them permanently low IgE levels were measured; in two patients the low IgE level

Table II

Quantitative relations of serum immunoglobulins in SLE patients with low and high IgE levels

	Average concentration of immunoglobulins ± SD (g/l)	
	IgE level < 100 kU/l	IgE level > 295 kU/l
IgG	16.96 ± 6.07	17.85 ± 3.56
IgA	2.61 ± 2.20	2.71 ± 1.29
IgM	1.18 ± 0.49	1.53 ± 1.39
n	55	21

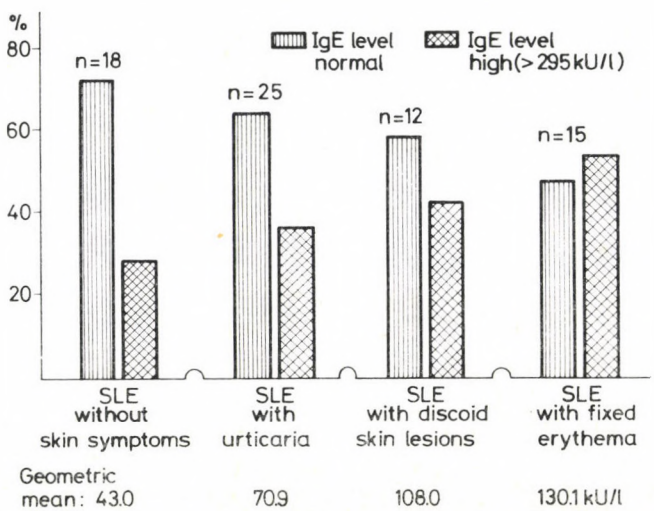

Fig. 2. Percentage of normal and very high serum IgE levels in SLE patients without and with skin symptoms

was combined with a lack of IgA (Table III). A permanently high IgE level was found in seven patients and in three patients this was associated with a permanently high IgA level.

Table III

Serum IgE level in SLE patients, follow-up examinations

IgE level	Permanently low	Permanently high	Fluctuating
(kU/l)	0.5—100	3000—1000	0.5—1000
n	23	7*	16**

* permanently high IgA level in 3 patients
** allergic history in 14 patients

The serum IgE level of 16 patients varied within wide limits and the variations did not show any correlation with activity of the disease. When looking for the explanation of this phenomenon, it was found that atopy or (more often) drug allergy was mentioned in the history of all but two of the patients. Among the 30 patients with a steady IgE level, 12 patients were allergic.

Out of the 46 SLE patients participating in the follow-up examinations, allergic phenomena were found in 26 patients (Table IV) in the form of exanthema, urticariform skin symptoms, rarely allergic rhinitis and angioneurotic oedema (we had no asthma patients). Some drug may have been the exciting agent in 20 cases, pollen or house dust in 2 cases, parasitic infestation in 1 case and no exogenous cause could be detected in 3 cases. In the allergic SLE patients the mean variation of the IgE level was found to be significantly higher than in the non-allergic patients. In some cases the appearance of allergic phenomena showed a correlation with the increase of the IgE level.

Table IV

Serum IgE level in allergic SLE patients, follow-up examinations

Patients group	Arithmetic mean of difference of *extreme values $\pm$ SD	Geometric mean of difference $\pm$ gSE
Allergic group n = 26	213.0 $\pm$ 287.5 NS	67.1 $\pm$ 1.41 $p < 0.05$
Non-allergic group n = 20	92.8 $\pm$ 150.8	12.6 $\pm$ 2.22

* extreme value: maximum and minimum IgE concentration measured in the same patient (kU/l)

Discussion

In systemic autoimmune disease entities including SLE IgE may be in excess due to the increased antibody production of the patients and the chronic course of the disease [5, 8, 16]. Some authors attribute a subordinate [14], others a significant role [3] to IgE in the pathomechanism of autoimmune diseases. The high concentration of serum immunoglobulins may be an important sign of activation of the disease [5], but it does not always unambiguously refers to the role played by a given immunoglobulin in the course of autoimmune disease.

In aggreement with other authors [9, 15], we have observed a wide scattering of the serum IgE content and so we assumed that an increased IgE level in SLE was a sign of the clinical activation of the disease in many patients. Still, as an expressed increase does not appear in every patient, the diagnostic value of a high serum IgE level is limited.

The production of IgE more or less differs from that of other immunoglobulins. At the same time, in some cases we have observed a lack of IgA in the presence of a permanently low IgE level as well as a high IgA concentration in the presence of a permanently high IgE level. This observation supports the theory of a genetically determined regulation of IgE production [12], and raises the possibility of regulatory disorders being present [1].

We found higher IgE levels in the background of fixed erythema accompanied with basic membrane degeneration and in SLE urticaria, similar in appearance to an allergic skin manifestation. Atopy occurred rarely, while drug allergy very often in our SLE patients. Goldman et al. [6] measured low IgE concentrations in the blood of allergic SLE patients. During our follow-up examinations of allergic patients we observed a capricious fluctuation of the IgE level. These data support the idea of an independent genetic regulation of allergen-specific IgE production [12], and at the same time they indicate a regulatory disorder of allergen-specific IgE production in SLE. On the other hand, all this may refer to the fact that it is not reagin-dependent reactions that can be found in the foreground of the clinical picture at the time of activation of the basic disease and of the appearance of local and systemic symptoms.

Several authors have raised the possible role of immune complexes containing IgE in the induction of diseases associated with immune complexes [3, 4, 8, 11]. It might be assumed that IgE antibodies, produced during the course of SLE like other antibodies, would develop circulating immune complexes causing systemic and local tissue damage. The local consequences of reagin-dependent reactions induced by immune complexes containing IgE specifically linking to the surface of basophils and mast cells may manifest themselves in an typical form in some cases, in so far as the vasoactive mediator

materials forming the main link of the reaction chain are consumed for the deposition of locally accumulated immune complexes [4].

As to the role of IgE antibody in immune complex diseases there are several alternatives; the question will have to be settled experimentally. In the present study we have only attempted to find an explanation for the changes of the IgE level in SLE from the clinical point of view.

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Cardiology

THE CLINICAL VALUE OF THE HIS-BUNDLE ELECTROGRAM IN INTRAVENTRICULAR CONDUCTION DEFECTS

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A study concerned with the clinical significance of the His-bundle ECG was carried out in association with upper atrial stimulation in 20 patients with intraventricular conduction defect. On the ground of the results the His-bundle ECG is regarded as a procedure of diagnostic value in intraventricular conduction defects by contributing to the accuracy of information on the severity and extent of the lesion. In the framework of published observations, including the present findings. questions relating to prognosis and to the necessity for pacing in bifascicular block are discussed.

Keywords: bifascicular block, His-bundle ECG, H-V interval, atrioventricular block

Abbreviations: IVDC: intraventricular conduction defect; HPS: His-Tawara–Purkinje system; HBE: His-bundle ECG; PA interval: intraatrial conduction time; AH-interval: A-V nodal conduction time; H-V interval: conduction time distally to the His-bundle

Introduction

The various intraventricular conduction defects (IVCD) may affect one or more sections of the His-Tawara-Purkinje system (HPS), in other words, the block may involve the bundle of His, the right bundle of Tawara, the stem of the left bundle of Tawara, its anterior or posterior fascicle, and the peripheral Purkinje-system [14, 15].

The various types of bundle and fascicular block may be diagnosed by the conventional 12-lead surface ECG. The form, width and axis position of the QRS-complex provide valuable diagnostic information on IVCD, but the traditional ECG lacks the necessary accuracy for any closer definition either of the degree of the conduction disturbance, or of the number and extent of the lesions in case if IVCD involving the HPS.

It has been reported in recent years that the His-bundle ECG (HBE) greatly contributes to the diagnostic accuracy in IVCD involving the HPS,

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and even allows to identify lesions eluding diagnosis by the traditional surface-ECG leads [2, 3, 7, 16, 17, 18, 19, 20, 21, 22, 23, 26, 28, 29, 30, 31].

Theoretically, a prolonged H-V interval of the HBE, in the presence of a bundle-branch block, predicts the progression of the conduction disorder to complete AV-block. Production of complete heart block and sudden death preceded by these signs have been reported by numerous authors [10, 18, 25, 29]. The significance of the H-V interval in the diagnosis of IVCD is, however, still open to controversy and requires further electrophysiological and clinical evidence. The present study has been concerned with the significance of the H-V interval in cases of bundle-branch block.

Patients and methods

In the period 1977/78 100 HBE were performed in our Institute. The investigation was prompted in the majority of the cases by sick sinus syndrome, WPW and LGL syndromes, supraventricular tachycardia, atrioventricular and intraventricular conduction disturbances [32]. This report is confined to those cases in which the presence of IVCD was accessible to ECG diggnosis. The HBE was recorded under conditions described earlier [30, 31].

Upper atrial stimulation was applied, subsequently the bundle of His was stimulated in order to bring the His-bundle potential into display [21]. Exhaustion of the conduction system by means of stimulation of increasing frequency (100–180/min) allowed to study the behaviour of the AV-node (A-H-interval) and of the His-Purkinje–Tawara system (H-V interval) [5]. The H-V interval was measured from the peak of the H-deflection to the earliest QRS-deflection appearing on the simultaneous ECG-tracing, 2 or 3 surface-ECG-leads being recorded simultaneously with the HBE. In order to study the H-V-interval more closely, 0.02 mg/kg body-weight of atropin was administered intravenously prior to atrial stimulation, with the aim of eliminating the balancing function of the AV-node [23]. After abrupt discontinuance of upper atrial stimulation the sinus node recovery time (SNRT) was measured together with its corrected value. In a few cases 0.5 to 1 mg/kg body weight of Ajmalin was administered i.v. for the study of the behaviour of the H-V-interval [8, 27]. The tracings were registered by a direct-writing Siemens–Elema 1600 or a Hellige 363-type at a paper speed of 25–50–100 mm/sec.

Results

Twenty HBE had been prompted by clinical manifestations associated with IVCD. The patients ranged in age from 11 to 66 years. Distribution of the various types of IVCD and the values of the electrophysiological parameters are shown in Table I. The IVCD were associated with AV conduction disturbances in 6, by sino-auricular block in 2 cases. The presenting symptoms were dizziness in 6, syncope in 4, palpitation in 5, weakness in 3 cases, bradycardia in one case. A patient with right bundle-branch block + lst degree AV-block, in whom periodic 3rd degree AV-block had been noted earlier, had now no complaints. In the present cases the P-A interval ranged from 25 to 50 ms, the A-H-interval from 50 to 120 ms and the H-V interval from 35 to 255 ms. The H-V-interval was abnormal in 7 cases. In 3 of the 4 patients with syncope it was abnormally increased. Its excessive increase to 255 and 155 ms in two patients with left bundle-branch block (LBBB) suffering from vertigo confirmed

the diagnosis of a bilateral bundle injury. An increase in the H-V interval by more than 5 ms was demonstrable in response to upper atrial stimulation in 4 cases, to stimulation under the effect of atropine in 9 cases. Two patients of the latter group developed a block distal to the bundle of His, one patient an intra-His block. In the majority, lengthening of the A-H interval was elicited by stimulation (100 to 150/min), no such response having been obtained in 4 cases. The SNRT ranged from 650 to 8000 ms, exceeding the normal value in 5 cases. A permanent pacemaker was implanted in 7 cases, temporary pacing was required in one case (case No. 20) because of asystole subsequent to ventricular fibrillation. In 4 of these cases bilateral bundle branch block, in 3 cases, the sick sinus syndrome had been the primary factor responsible for the symptoms. In the following some illustrative cases are reported, the respective serial numbers being listed in Table I.

Case 1. K. I., a female aged 61 was admitted with dizzines and fainting. The ECG was typical of a LBBB + 1st-degree AV-block. The HBE revealed an excessively prolonged H-V interval (255 ms) and a normal A-H interval (Fig. 1). A permanent pacemaker was implanted.

Case 4. S. K., a female, aged 48, had experienced recurrent episodes of momentary unconsciousness over the last weeks. The ECG revealed, in addition to an intermittent LBBB and LAHB, a frequently recurrent sino-auricular block. The intervals of the HBE, in both the LBBB as well as in the LAHB beats, were found normal (Fig. 2) and remained unchanged in response to stimulation. The SNRT attained, however, 3150 ms. After implantation of a permanent pacemaker she was discharged symptom-free.

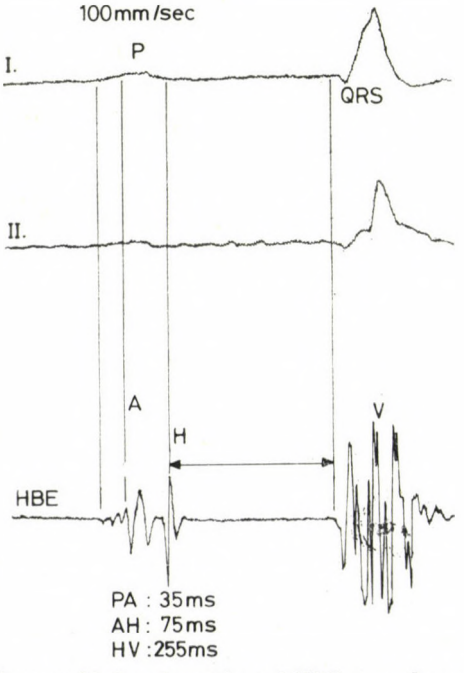

Fig. 1. K. L., 61-year-old female patient LBBB + prolonged H-V interval (incomplete bilateral bundle-branch block)

Table 1

Electrophysiologic data in patients with intraventricular conduction disorders

Serial No.	ECG	R-R	P-A	A-H	H-V	SNRT	Wenckebach point	H-V interval		PM implant
								stimulation 100—180 per min.	atropine + stimulation	
1.	LBBB + AV-I	860	35	75	255	1600	120	255	260	+
2.	LBBB + RBBB + premat. c.	800	50	50	35	1100	120	35	50	—
3.	RBBB + LAHB	1070	40	70	50	1180	100	50	60	—
4.	LBBB + LAHB + S-A bl.	1820	40	50	60	3150	100	60	60	+
5.	RBBB + LAHB + AV-b I.	760	40	120	70	1300	120	70	70	+
6.	RBBB + LAHB	680	50	70	50	8000	120	50	60	+
7.	Interm. LBBB + LAHB	700	45	75	45	1300	120	45	45	—
8.	RBBB + AV-bl. I. + premat. v. c.	950	40	170	50	1110	100	50	50	—
9.	LBBB + interm. LAHB	700	50	50	155	2000	—	155	155	+
10.	RBBB + LAHB	780	40	80	55	780	100	55	AV-bl. dist. to H 55	—
11.	LBBB	660	35	60	45	650	—	50	occasional bl. dist. to H	—
12.	LBBB	780	40	50	45	650	120	50	Intra-His-bl.	—
13.	LAHB + RBBB + AV-b I.	780	50	75	115	1400	150	115	120	+
14.	LBBB + S-A-bl.	1260	25	100	75	2800	120	75	80	+
15.	RBBB	760	40	130	40	1200	120	40	40	—
16.	RBBB + AV-bl. I. + interm. AV-bl. III.	780	50	170	45	1150	100	45	45	—
17.	LAHB + AV-bl. I.	900	50	110	65	1200	150	70	70	—
18.	LBBB	800	50	30	50	1200	—	50	50	—
19.	LAHB	660	35	80	45	650	—	50	45	—
20.	interm. LAHB + LAHB + prem. atr. contr.	680	40	70	40	820	retrograde conduction	40	—	temporary

Case 12. S. P., a female aged 56, had been on digitalis and diuretics because of circulatory failure for years, and had been noticing occasional slowing of heart rate. The surface ECG showed a typical LBBB with normal AV-conduction. The intervals of the HBE were normal. High right atrial stimulation at 125/min failed to elicit any change in the H-V interval, but after i.v. atropine, production of an intra-His block was registered during stimulation (176/min). No permanent pacing was required (Fig. 3).

Case 13. P. T., a female patient aged 65, hypertensive for 14 years, was admitted for recurrent brief spells of unconciousness experienced lately. The surface leads revealed, in addition to a LAHB + RBBB a 1st degree AV-block with the PQ measuring 0.24 sec. The H-V interval of the HBE was prolonged (115 ms), thus confirming that, in addition to the bifascicular block, conduction by the posterior, fascicle, was also affected (trifascicular block). The H-V interval remained unchanged upon upper atrial stimulation (Fig. 4). After implantation of a permanent pacemaker the patient was discharged symptom-free.

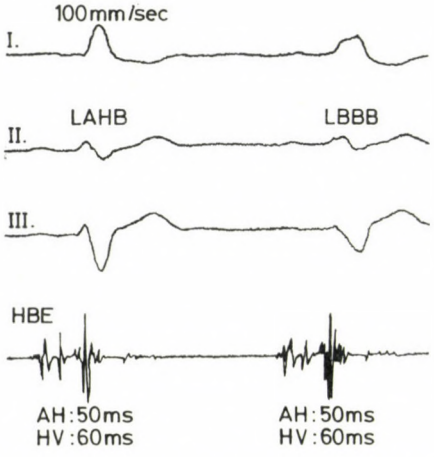

Fig. 2. S. H., 48-year-old female patient. Intermittent LAHB and LBBB (normal time-intervals)

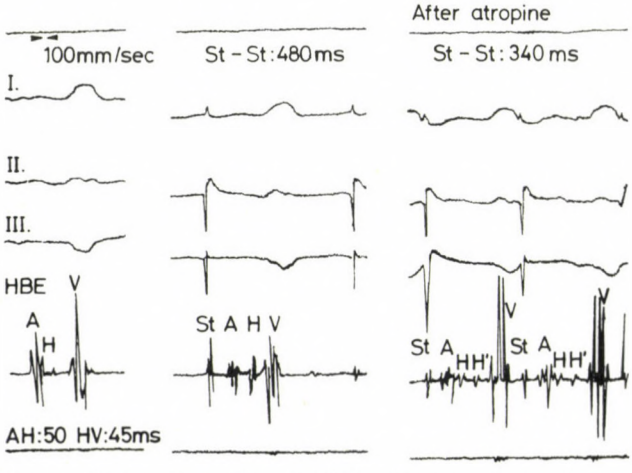

Fig. 3. S. P., 56-year-old female patient LBBB with normal time-intervals. Upper atrial stimulation under the effect of i.v. atropine elicits an intra-His block

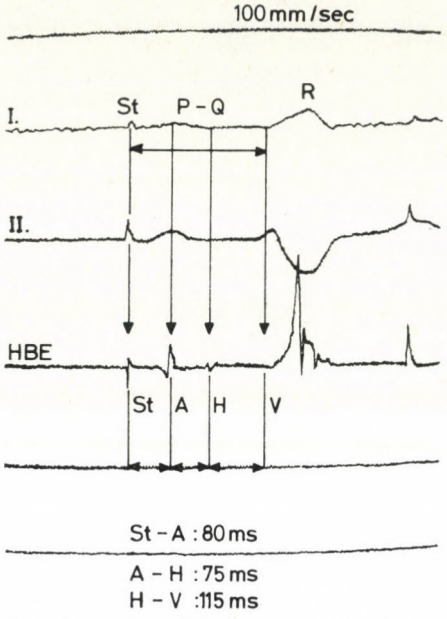

Fig. 4. P. T., 65-year-old female patient. Upper atrial stimulation leaves the AH and HV intervals unchanged

Discussion

While 6 to 10% of patients with bifascicular block are liable to develop a complete AV-block with Adams-Stokes attacks at some later time [4, 12, 13, 24, 26), the majority remain free from this complication [3, 7, 32]. Delimitation of a homogeneous group in which this is bound to occur is, obviously, impossible on the basis of conventional leads. The HBE, whose H-V interval is accessible to accurate measurements, does, however, allow to separate the two groups, according to whether the conduction time of the still functioning branch is normal or prolonged. The significance of this lies in the possibility of predicting the clinical course, possibly of recommending arteficial pacing beforehand, so as to prevent the production of a complete heart-block and of the Adams-Stokes syndrome [11]. A close electrophysiological investigation of this kind has also the advantage of making unnecessary pacing avoidable if it shows a normal profile of the conduction system in patients with bifascicular or bundle-branch block, experiencing spells of dizziness or syncope, particularly in advanced age. In the presence of a normal H-V interval the last-named symptoms point to a neurological or cerebrovascular, rather than to a cardiac, origin [6, 32]. In our IVCD patients the HBE has been of substantial diagnostic aid. The abnormal H-V interval supported the diagnosis of bilateral bundle lesion in 3 out of 4 patients with symptoms of syncope, and in 2 of these the

presence of the sick sinus syndrome was also confirmed on the ground of an abnormal SNRT.

Permanent pacing was applied in three cases, temporary pacing in the fourth case of the syncope-group. In another three cases (patients 1, 5, 9) bilateral bundle lesion, confirmed by an abnormally increased H-V interval, justified in accordance with the clinical symptoms the implantation of a permanent pacemaker. Exhaustion of the conduction system by atrial stimulation may disclose eventual concealed conduction defects [5, 17, 19, 21, 28]. In the majority of the present cases an increase in the A-H interval was demonstrable in response to upper atrial stimulation. The function of the conduction system distal to the bundle of His is difficult to study, owing to the screening role of the AV node. For this reason, stimulation failed to produce any significance change in the H-V interval. Atropine is, however, capable of inhibiting the balancing function of the AV node. A lengthening of the H-V interval was indeed demonstrable, in 9 of our cases in response to stimulation under the effect of atropine. In 2 of these, production of a block distal to the bundle of His (Cases Nos 9 and 11), and in one case (No. 12) an intra-His block was noted.

A classic bundle-branch block demonstrable by conventional ECG leads associated with other conduction disorders is a sign of extensive damage to the conduction system. It is, however, often impossible to tell from the ECG alone, whether the AV-conduction disorder associated with a bundle-branch block was reflecting a lesion of the AV-node, either one near the HPS, or one distal to the node of His. In 6 of the present cases a lst-degree AV-block associated with IVCD was shown by the surface ECG. On the evidence of the HBE the lesion responsible for the AV-block was distal to the bundle of His in 4 (Nos 1, 5, 13, 17), and proximal to it in in 2 (Nos 8, 16) cases. Similarly to the 1st-degree block, the 2nd-degree-block may also have a lesion of the conduction system proximal or distal to the bundle of His as its site of origin. It is the exact location of the lesion rather than its type, that determines the prognosis [17]. In our Case No. 9 2 : 1 AV-block in association with a LBBB was noted. On the evidence of the HBE, the site of the block was distal to the bundle of His. If in patients complaining of dizziness the HBE-intervals were found negative, unnecessary pacemaker implantation could be avoided, in view of the reliability of this diagnostic procedure. In Case No. 20 a concealed retrograde conduction of AV-junctional premature contractions of bigeminal arrangement had been responsible for a gradual increase in the A-H interval in the sinus beats following upon the premature contractions and thus, for the production of Wenckebach periodicity. A similar case by surface ECG has been reported by Damato and Lau [1], but to our knowledge before our Case No. 20 no observation of this uncommon rhythm disturbance identified by HBE has been published [33].

The length of the H-V interval in IVCD has been related by numerous authors to the production of complete heart block and to sudden death at some later time. Scheimann et al., following up 39 cases of bundle-branch block with H-V intervals longer than 75 ms for an average period of 18 months, noted the production of 2nd or 3rd-degree block in 7 cases; 13 patients died, 5 of them suddenly. In the 14 patients of Vera et al. [29] who had bundle-branch block in association with syncope, 2nd or 3rd-degree AV-block, the H-V interval was invariably longer than 65 ms. In the 10 patients of Narula et al. [17] who had LAHB + RBB in association with H-V intervals exceeding 55 ms, the mortality in a year was 61 percent. Permanent pacing of patients with asymptomatic bundle-branch block was advocated by these authors if the H-V interval was longer than 70 ms. Dhingra et al. [7] found H-V intervals over 80 ms in not more than 18 (5%) out of 388 patients with bundle-branch block. Production of a 2nd or 3rd-degree AV-block was confined to one (6%) of the patients during an average follow-up period of two years. The death figure in their patient material was 33%, as opposed to 61% reported by Narula et al. [17]. It has however to be noted that the study of Dhingra et al. [7] was prospective, the patients with confirmed AV-block or episodes of syncope associated with the bundle-branch block having been excluded at the onset. On the other hand, the figures of Scheimann et al. [25], Narula et al. [18] and Vera et al. [29] were based on retrospective studies with no selection on the basis of the criteria referred to above.

From the present observations the HBE emerges as a procedure suitable for the study of patients with IVCD. It should be performed in all cases in which IVCD are associated with dizziness, syncope or bradyarrhythmia. Abnormal electrophysiological findings, in particular a H-V interval exceeding 75 ms or an abnormal SNRT, call for preventive implantation of a pacemaker [11]. Negative HBE intervals in patients with bundle-branch block complaining of dizziness exclude the possibility of Adams-Stokes attacks and allows to avoid unnecessary pacing. Obviously, neurological investigations for the possible causes of the symptoms are required in these cases.

Periodic follow-up studies should be made in symptom free cases of bundle-branch block, but electrophysiological investigations are not necessary.

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Laboratory

PRODUCTION AND PROPERTIES OF ANTISERUM FOR RADIOIMMUNOASSAY OF SERUM CONJUGATED CHENODEOXYCHOLIC ACID AND ITS PRELIMINARY APPLICATION

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Sensitive tritiated radioimmunoassay was developed for conjugated chenodeoxycholic acid, using immunogen prepared by the mixed anhydride method. The obtained molar bile salt-BSA ratio in the immunogen was 19 : 1. The distinguishing features of the immunization procedure were a preliminary vaccination of the animal with antitubercular vaccine (VDS), and the administration of very small doses of immunogen (50 μg). Assay sensitivity for this bile salt fell in the picomole range with the standard curve extending from 1.5 to 150 pmol. Specificity of the antiserum was compared with that of the commercially available "Glycochenodeoxycholic acid RIA kit" (Nordiclab Oy, Oulu, Finland), and proved to be satisfactory. Fasting serum conjugated chenodeoxycholic acid concentration in 25 healthy subjects and 15 patients with cirrhosis was 0.63 μmol/l and 43.05 μmol/l, respectively. The assay was performed on unextracted sera.

Keywords: bile salt, radioimmunoassay, antiserum, conjugated chenodeoxycholic acid

Introduction

The interest in serum bile acid determination has considerably increased recent years. Of the blood tests used routinely to assess liver function, none is ideal. Only some of them can be used to measure hepatocyte function and even they are rather insensitive. No doubt, there is a need for better tests. The synthesis and excretion of bile belong to the fundamental functions of the hepatobiliary system. The hepatocytes synthesize bile acids by microsomal hydroxylation of cholesterol. A number of studies have shown that serum bile acid concentration is a sensitive indicator of liver function and that highly increased bile acid levels are seen in the sera of patients with hepatobiliary diseases [6, 9]. The early methods used for the measurement of serum bile acide were insensitive, time and money consuming [4, 8]. The problem was

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resolved when the first bile salt radioimmunoassay was reported by Simmonds et al. [14] allowing a sensitive assay of large numbers of samples. Although during the past ten years a great number of modifications of the above method was published [5, 7], the problem of insufficient specificity of the prepared antiserum still exists and the assay of sera requires time and labour consuming extractions.

The present study describes a rapid, sensitive radioimmunoassay of unextracted serum for chenodeoxycholic acid conjugates. Chenodeoxycholic acid was chosen because its serum concentration is the highest of all the bile acids.

Materials and methods

Bile acids were purchased from Sigma, P-L Biochemicals, Calbiochem; purity was analysed by HPLC, using VARIAN model 8500 liquid chromatograph equipped with VARIAN series 634 UV-spectrophotometer as a detector, HEWLETT-PACKARD 3380 A integrator, 25 cm × 4.6 mm I.D. KNAUER HPLC-column packed with LiChrosorb RP-18 5 μm. The solvent system was a mixture of HPLC-grade methanol (MERCK) — 0.005 mol/l KH_2PO_4 buffer (80 : 20, v/v), the pH was adjusted to 2.0 with concentrated H_3PO_4. Flow rate was 1.0 ml/min. UV detection was performed at 215 nm. ^{3}H-chenodeoxycholylglycine (specific activity 85.1 GBq/mmol) was obtained from New England Nuclear Co., Boston, Mass. Bovine serum albumin (fatty acid-free) was supplied by Sigma, Freund's complete adjuvant from Difco Laboratories Ltd. VDS (Salvioli Diffused Vaccine) was a gift of Ghimas, Spa, Bologna, Italy. Tri-N-butylamine and isobutylchloroformate were obtained from Fluka AG, Buchs, Switzerland, Scintillation liquid was a Triton X-100-(Serva)-based fluid. Other chemicals used in the experiments were of analytical grade.

Preparation of immunogen. Bile acid-albumin immunogen was prepared by covalently coupling bovine serum albumin (BSA) to hapten (chenodeoxycholylglycine) by the mixed anhydride technique [3]. The coupling procedure was a modification using fatty acid-free bovine serum albumin. The albumin-bile acid compound was separated from unconjugated bile acid by dialysis against 0.01 mol/l phosphate buffer pH 7.4 at 4 °C for 72 h. ^{3}H-labelled bile acid was added to determine the conjugation efficiency. The radioactive content of an aliquot (1%, v/v) of the initial reaction mixture was determined with the aliquot (5% v/v) of the dialysed fraction, and the binding was calculated. Immunogen was diluted to protein concentration of 100 μg/ml with 0.9% sodium chloride and emulsified with an equal volume of Freund's complete adjuvant until a stiff water in oil emulsion was obtained.

Preparation of antiserum. Three rabbits were injected intradermally with 0.75 mg of VDS on the inside of the thigh. Immunization began 6 weeks after vaccination. Intradermal injections were given to 10 abdominal sites at a dose of 50 μg of immunogen per rabbit. This treatment was repeated 3 times in weekly intervals and then a booster dose (50 μg) was given monthly.

The antigenic response of the immunized animals was determined by the Ouchterlony double-diffusion technique and dilution of the serum that would bind 50% of 10 000 cpm ^{3}H-chenodeoxycholylglycine. The highest titres were those for antisera collected 20 weeks after the initial immunization. After separation, sera were stored at −20 °C and a suitable dilution was prepared when required.

Assay procedure. Antiserum, tracer and samples were diluted with 0.02 mol/l phosphate buffer pH 7.4 containing 0.6% sodium chloride, 0.1% bovine serum albumin and 0.1% sodium azide. The tracer solution contained about 10 000 cpm/0.1 ml ^{3}H-chenodeoxycholylglycine in buffer solution. Antiserum was diluted with albumin buffer so that the final dilution used in radioimmunoassay gave 50% bound antibody. The standard curve consisted of 0.1 ml bile acid solution containing 1.5, 5, 15, 50, 150 pmol. First, 0.2 ml buffer solution, 0.1 ml standard or the unknown serum (diluted 1 : 10 or 1 : 50, according to the supposed concentration), 0.1 ml tracer solution and 0.1 ml diluted antiserum were added to the test tubes. The tubes were mixed on Vortex and left at room temperature for 1 hour. Saturated ammonium sulphate (0.5 ml) was added to the tubes, thoroughly mixed, equilibrated at 4 °C for 45 minutes and

centrifugated at 1500 g for 20 minutes at 4 °C. An aliquot of the supernatant (0.5 ml) was mixed with 7 ml of scintillation mixture. Samples were counted with a Packard Tri-Carb Model 3375, the quenching proved to be constant.

Test sera were obtained after a 12 h fast. Blood was allowed to clot at room temperature, and the serum was separated by centrifugation and immediately deep frozen until assayed. Results were expressed as means ± S.E.M. The significance of difference of mean values was tested by Student's *t* test.

Results

The mixed anhydride reaction gave a hapten (chenodeoxycholylglycine)-protein ratio of 19 : 1.

Figure 1 shows the antigenic response determined by Ouchterlony double-diffusion technique. Five weeks after the beginning of immunization, the serum obtained from rabbits mixed with bovine serum albumin in excess and centrifuged (to remove anti-BSA antibodies) showed no precipitation by diffusion against bovine serum albumin in the double-diffusion test whereas a single precipitation line was obtained by diffusion against chenodeoxycholylgylcine-bovine serum albumin conjugate.

Figure 2 shows the highest antibody dilution capable of binding 50% of a tracer dose (10 000 cpm/tube) of ^{3}H-chenodeoxycholylglycine plotted against the time of immunization, in three rabbits. 26 weeks after vaccination by VDS, antibody titres were 1/2500, 1/3000 and 1/3500, respectively.

Cross-reactivity of antisera to other 13 bile salts was determined according to Abraham [1]. Table I illustrates the specificity of our antiserum in comparison with commercially available RIA kit antiserum examined by Nordiclab [7] and Källberg et al. [5]. The specificity of antiserum determined by the manufacturer and Källberg et al. was not the same. Our antiserum and Nordiclab's antiserum (determined by Källberg) had almost the same sensitivity to both conjugates of chenodeoxycholic acid, but a lower sensitivity

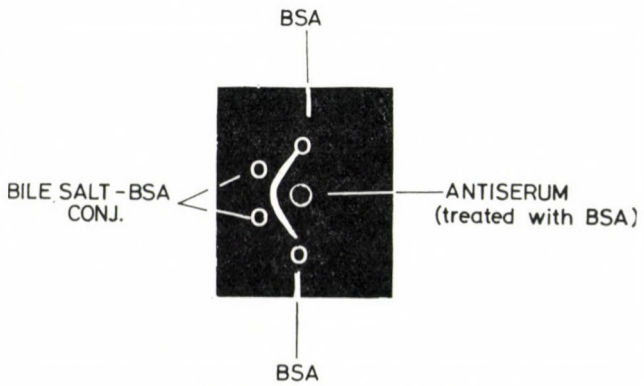

Fig. 1. Ouchterlony double-diffusion test of bovine serum albumin and chenodeoxycholylglycine-bovine serum albumin compound against antiserum to chenodeoxycholylglycine

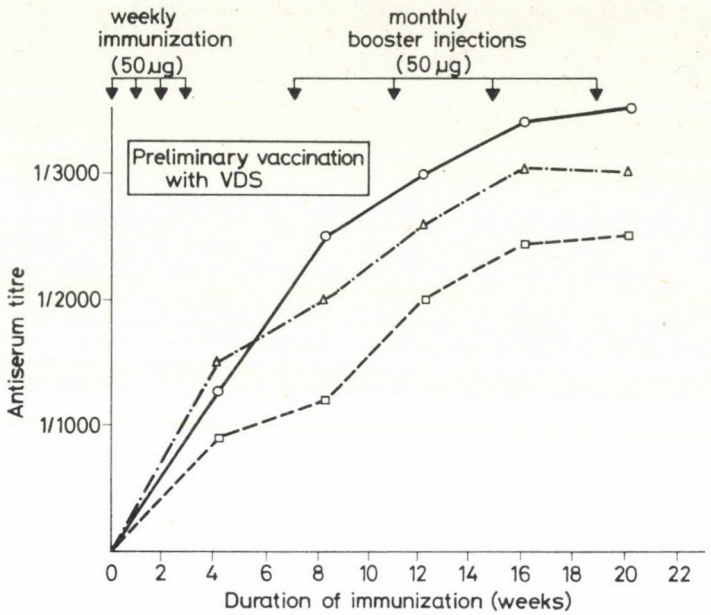

Fig. 2. Time course of antibody titre in rabbits after immunization with chenodeoxycholylglycine

Table I

Relative cross-reactivity (percent) of different bile acids with two antisera

Bile acid	Cross-reactivity, percent		
	Our antiserum	Antiserum of Nordiclab RIA kit examined by Manufact. Källberg et al. [5]	
Chenodeoxycholylglycine	100	100	100
Chenodeoxycholyltaurine	90	50	90
Chenodeoxycholic acid	20	100	33
Cholic acid	<0.1	0.1	0.4
Cholylglycine	0.4	0.8	0.3
Cholyltaurine	0.5	0.4	0.6
Deoxycholic acid	<0.1	<0.1	—*
Deoxycholylglycine	0.1	<0.1	—*
Deoxycholyltaurine	<0.1	<0.1	—*
Lithocholic acid	0.3	0.6	—*
Lithocholylglycine	0.5	8.0	—*
Lithocholyltaurine	0.5	1.5	—*
Sulphated lithocholylglycine	<0.1	<0.1	—*

* Not examined

to unconjugated chenodeoxycholic acid and low sensitivity to cholic acid and its conjugates. Cross-reactivity of our antiserum to other bile salts e.g. lithocholylglycine and lithocholyltaurine seemed to have some advantage over the "Glycochenodeoxycholic acid RIA kit" antiserum according to the data given by the manufacturer.

Figure 3 shows the standard curve for chenodeoxycholylglycine. Each point represents the mean ± SD calculated from five triplicate analyses. Thus, the standard curve range was set at 1.5–150 pmol.

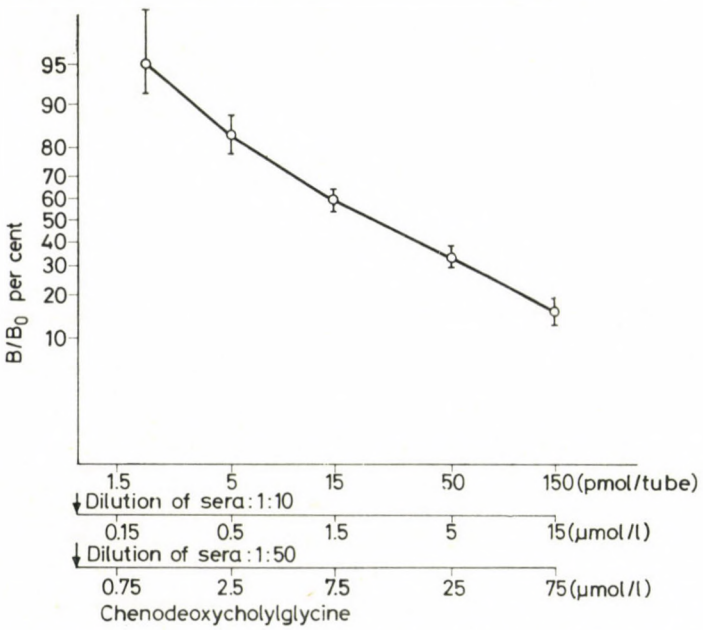

Fig. 3. Standard radioimmunoassay curve for chenodeoxycholylglycine. The curve reflects a logit transformation of B/B_0 vs. log dose concentration

The least detectable amount of chenodeoxycholylglycine, defined as mean + 2 S.E.M. of the value for zero dose, was 1.5 pmol/tube. The intra-assay precision at a low serum concentration of chenodeoxycholylglycine (0.6 μmol/l) proved to be 8.5% and at a higher serum concentration (20 μmol/l) 11.0% (coefficient of variation). The interassay precision determined by subjecting the samples to 6 different assays, were 13.4% and 14.7% (coefficient of variation). Recovery estimated by adding various amounts (5, 15, 50, 100 pmol) of chenodeoxycholylglycine to pooled serum ranged between 91 and 108%.

Serum values of 25 control subjects with no evidence of liver disease and 15 patients with cirrhosis confirmed by liver biopsy are shown in Table II. Mean concentration were 0.63 μmol/l and 43.05 μmol/l, respectively.

Table II

Serum concentration of chenodeoxycholylglycine in normal subjects and patients with cirrhosis (mean ± S.E.M.)

	Chenodeoxycholyl-glycine μmol/l
Controls (n = 25)	0.63 ±0.06
Cirrhosis (n = 15)	43.05* ±9.96
*P	<0.001

Discussion

The advantage of using fatty acid-free bovine serum albumin made the mixed anhydride reaction attractive and gave a higher hapten-protein ratio than the 12 : 1 observed by the other authors [10, 11], who used the mixed anhydride procedure too. The question of the optimum bile salt-protein ratio has not been settled. It is however, generally accepted that it is favourable if among 59 terminal amino groups of lysine residues of BSA, 15–30 could be conjugated to bile acids [13].

In the present work, we applied a pretreatment of the animals with a non-specific adjuvant (VDS). This material contains tuberculosis bacilli of human and bovine origin, killed and mixed with hyaluronidase to facilitate spreading from the site of injection to the lymph nodes. Bachiega et al. [2] and Roda et al. [12] stressed the beneficial result of this pre-inoculation. Probably, the effect differs from Freund's complete adjuvant and plays a role mainly in the first part of immunization. Another important factor in our immunization schedule was the use of 50 μg of antigen in contrast to other authors [10, 14], who applied 1–2 mg of the antigen. The result of these immunization conditions was that the titre and specificity of our antisera proved to be sufficient. Higher titre as reported by other authors [12] might be due to the application of lower tracer activity.

Radioimmunoassay reports agree that antisera to bile salts are specific for the bile salt structure but show variable specificity to other bile salts. Our antisera, similarly as those of others [7, 14], cannot discriminate between glycine and taurine conjugates, but affinity to unconjugated bile acids is only 20% of that for conjugates, moreover the free chenodeoxycholic acid is only a small part of the serum total chenodeoxycholic acid content. Therefore, we call our method "RIA for conjugated chenodeoxycholic acid".

The sensitivity of our RIA allows to determine picomole amounts in serum. As bile acids are present in nanomole/ml concentrations, the assay is convenient for measuring normal as well as pathologically increased values.

The commercially available RIA kit has the disadvantage of needing a previous extraction of bile acid with ethanol. Using our assay conditions no serum effects were found, so that extraction of serum prior to assay was unnecessary.

According to the literature [7, 14] in fasting normal subjects the serum levels of conjugated chenodeoxycholic acids are less than 1 μmol/l. In hepatobiliary disease the concentration of bile acids is significantly higher than in the controls several hundred fold increases may be found in serious cases. Normal sera were diluted 1 : 50 with assay buffer, to bring the concentration to the range of the standard curve. Our data for the fasting bile acid concentration showed a significant difference between the means of the two groups. With one exception, in the control group all bile acid values were below 1 μmol/l. In the cirrhotic group all patients showed increased concentrations of conjugated chenodeoxycholic acid. Since the group was heterogeneous, it is suggested that the bile acid level may be influenced by the stage of cirrhosis. Further studies of a large number of samples are required to elucidate the biologic variability and to obtain information about physiological and pathological events.

Acknowledgements

This work was supported by the Hungarian Ministry of Health.

We are indebted to Drs P. Ungár and L. Romics (National Institute of Rheumatology and Physiotherapy) for sera of patients with cirrhosis. Thanks are due to Mrs. O. György and Miss Zs. Suhajda for skilful technical assistance.

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Acta Medica Hungarica, Volume 42 (1—2), pp. 85—99 (1985)

Book reviews

Cancer Mortality by Occupation and Class 1951–1971 Logan, W. P. D. IARC Scientific Publications No. 36.

International Agency for Research on Cancer, Lyon, 1982. 253 pages, with numerous tables and figures. Price: Sw. fr. 60.—

The latest volume issued by the International Agency for Research on Cancer (IARC) is of the usual standard, both in content and in production. By providing an impressive body of information it offers valuable aid to all those engaged in cancer research. The various relationships and data relating to distribution and prevalence are made accessible to the reader by tables of clear arrangement, accompanied by short, instructive explanatory notes concentrating on essentials.

The are six chapters. The Introduction and Chapter 1 offer general explanations. Chapter 2 entitled "The present study: classification and indices", falls into the following subdivisions: Social classes and sub-classes; Socio-economic groups; Occupation orders; Occupation units; Classification of causes of death (on the ground of the International Classification of Diseases of WHO; Mortality indices), the indices and formulae necessary for the calculations. Chapter 3 covers the second half of the nineteenth century, giving a brief survery of the main events. Chapter 4 a Commentary on the tables 1911–1977, which occupies 71 pages, thus making up a substantial part of the book, deals with cancer mortality and with its various aspects organ by organ, on the basis of the International Classification of Diseases of WHO. Chapter 5 on socio-economic relationships, is of particular interest by covering the situation in Australia, Finland, France, Hong-Kong, Japan, Northern Ireland, Norway, Scotland, USA. Chapter 6 includes a summary, recommendations, as also a classified bibliography with 200 references. There are five appendices with tables.

L. Cselkó

Cancer Incidence in the USSR, 74 pages: published as the 48th volume of the IARC, Scientific Publications series No. 48. (in English)

Price: Sw. fr. 30.— IARC, WHO

The book is a publication of the Ministry of Health of the USSR, Department of Medical Statistics, Petrov Research Institute of Oncology, Leningrad, and of the International Agency for Research on Cancer (IARC) issued as a supplement of the internationally well-known

IARC Cancer Incidence in Five Continents. The editors of the version published in the USSR are, N. P. Napalkov, G. F. Tserkovny and V. M. Merabishvili. The editors of the book published in Lyon in the English language are, D. M. Parkin, M. Smans and C. S. Muir. Vol. 3. The aim of the publication — as drafted in the preface by the director of IARC — is to make the data of cancer patterns in the USSR available to scientists unable to read the original Russian literature. The book is divided into 6 chapters. The first, introductory chapter contains data on the geographical features, population and medical facilities of the USSR. Chapter 2 familiarizes the registration technique of cancer diseases. The registration of cancer cases and mortality is the responsibility of the oncological dispensaries serving catchment areas throughout the Soviet Union. The registration is based on a follow-up card introduced in 1975-76, containing 31 items. The 8th version of the International Classification of Diseases (ICD) is used for the grouping of cancer cases. Great importance is attributed to the grouping of collected data according to reliability (indicating the data based on histological verification and the death certificate). These are analysed in Chapter 3. The next chapter provides data on age--specific morbidity rates by sex and tumour sites for the 15 republics of the USSR. The editors present the data of the tables, broken down according to republics, in a layout identical to that used in Volume 3 of Cancer Incidence in Five Continent, to facilitate on International comparison. Chapter 5 comprises age-standardized rates concerning the 15 republics of the USSR. Here the publication of data differs from the manner used in Cancer Incidence in Five Continents, where the date are calculated using five-year age groups. The data in the present book were complied according to ten-year age groups. Chapter 6 is the most interesting one, in which — according to the various regions of the USSR — the editors demonstrate differences in the incidence of the various cancer diseases, as well as the changes observable in the frequency of occurrence since 1965. It is interesting that the incidence of oral and pharyngeal cancer is higher in the Central Asian republics and Azerbaijan than elsewhere. There are also great variations between the different republics in the rate for oesophageal cancer. The data concerning the incidence of stomach and lung cancer indicate important changes. While in 1969 and 1971, stomach cancer was the most frequent in both sexes in the Soviet Union, in 1980 lung cancer in males had the highest incidence. An increasing tendency was observed regarding breast cancer cases between 1965–1980, particularly in the Baltic republics. Related to the whole Soviet Union, however, the incidence of breast cancer is still lower than in the Western countries, and its incidence is the lowest in the Asian republics, where breast cancer is less frequent than in Japan.

It can be seen, therefore, that this book is a repository of extremely abundant material, compiled with attentive critique. The publication is useful for epidemiologists, specialists in the field of public health and oncologists.

K. Lapis

The Promotion and Development of Traditional Medicine

Technical Report Series, 622. WHO, Geneva 1978. 41 pages, 2 figures. Price: Sw. fr. 5.—

A WHO Meeting on the Promotion and Development of Traditional Medicine was held in Geneva from 28 November to 2 December, 1977, in conformity with the following agenda:

— Traditional medicine in health care Reasons for the promotion of traditional medicine
— Utilization of traditional medicine in national health care system
— Integration of traditional medicine and modern medicine

— Manpower development for traditional medicine
— Research promotion and development in traditional medicine
— Recommendations.

In order to discuss the actual subjects, an agreement had to be reached on the basic definitions such as "traditional medicine", "health care system" and on their integration.

Some of the subjects at issue receive a particularly thorough coverage, first of all, the nature, goal and scope of traditional medicine, illustrated by accounts from some member countries (Sri Lanka, Sudan, Egypt, Ghana, India). The possibilities, problems, ways and means of integrating traditional medicine and modern medicine are considered in all their aspects.

Discussion of manpower development, exemplified by the Cameroonian model, is also of interest. The part on research promotion and development is based on research promotion and development is based on experience in three countries (Mexico, Nigeria, China) and points to the possibilities of traditional medicine in various fields, such as human reproduction, tropical medicine (with the exception of malaria, schistosomiasis, filiariasis, leprosy, trypanosomiasis, leishmaniasis), drug dependence (where acupuncture has been found of potential benefit), cancer chemoteraphy, rheumatoid arthritis and some other diseases (cardiovascular diseases, diabetes, burns, acute abdominal ailments, bone fractures, kidney stones, gallstones).

The book closes with theoretically well-founded practical recommendations.

L. Cselkó

Vaccination Certificate Requirements for International Travel and Health Advice to Travellers 1983.

WHO, Geneva 1983. 70 pages with 1 figure, 2 tables and 3 maps. Price: Sw. fr. 12.—

The latest publication in this annual series is almost completely identical, even as concerns the number of its pages, with its predecessor. The introductory notes on the necessity of international vaccination certificates (IVC) are followed by information on the following topics: IVC requirements in the various countries, risk factors grouped according to continents and countries, and practical recommendations in the interest of prevention. The data reflect the situation of 1 January, 1983.

In the volume 210 countries are represented. The situation has changed since the last year as in 65 countries no vaccination certificates are required. As to the other 145 countries, IVC for yellow fever is required in 114, for yellow fever + cholera in 29, for cholera in 2 countries. Vaccination certificates for typhoid, smallpox and poliomyelitis are no longer required in any of the countries. On the other hand, accounts of the hazard and/or incidence of malaria have come this year from 102 countries.

The closing chapter on the prevention of diseases is very useful and more exhaustive than in the previous publications (food- and water-borne diseases, rabies, tetanus, bites by insects and other animals, chemoprophylaxis of malaria, sexually transmitted diseases, etc.).

The volume contains excellent tables and maps showing the distribution of diseases.

L. Cselkó

International Health Regulation (1969) Third Annotated Edition.

WHO, Geneva 1984. 79 pages. Price: Sw. fr. 11.—

The International Health Regulations adopted by the second World Health Assembly on 21 July, 1969, represent a revised and consolidated version of the previous International Sanitary Regulations.

The publication includes 9 sections and 4 appendices. Some of the sections are subdivided into chapters.

Section I gives definitions. In Section II notifications and epidemiological information are found. Section III deals with health organization. Section V gives the regulations for plague, yellow fever and cholera. The strict measures concerning plague and yellow fever are given in close detail, in the framework of 11 papers each. The measures against cholera are dealt with in 4 papers. It is emphasized that these three diseases represent a major threat and call, by this fact, for permanent alertness. Sections VI–IX provide information on health documents, charges, various provisions. In Appendices 1–4 we find forms for various declarations and certificates, e.g. vaccination against yellow fever, revaccination, Maritime Declaration of Health and the health part of the Aircraft General Declaration. The sanitary standards of air and maritime traffic are found in the subsequent appendices. The volume is very well indexed.

L. Cselkó

Seventh General Programme of Work, covering the period 1984–1989.

WHO, Geneva. 153 pages. Price: Sw. fr. 8.—

This publication, the 8th volume in the series "Health for All", gives a programme in the form of a close time-table of the essential tasks.

Section 1 contains introductory notes on the general lines, ends and means of the programme. Section 2 takes a retrospective look at the results of the previous programme. Section 3 gives a summary of the global strategy of Health for All. Sections 4–5 provide information on the roles, processes, functions, structures, general programmes and framework of WHO. Section 6 outlines the main trends of the programmes and determines the priorities. Section 7 presents a programme outline in accordance with the classified list of programmes, in a practical form. In chapter A of this section questions of management and coordination are discussed. Chapter B on the infrastructure of health systems, concentrates on the development and organization of health system based on primary health care and on information and healt education of the population. In Chapter C the following topics are dealth with in the framework of health seciences and technology: research promotion and development; general health protection; protection and promotion of mental and environmental health; disease prevention and control. In Chapter D the tasks involved by programme support are formulated in close detail. In Sections 8 and 9 implementation of the programme including monitoring and evaluation, is outlined, Section 10 gives a brief summary. There is an appendix and an index at the end of the volume.

L. Cselkó

Plan of Action for Implementation of the Global Strategy for Health for All and index of the "Health for All" series Nos 1–7.

WHO, Geneva. 1982. 55 pages. Price: Sw. fr. 6.—

This brochure has been published as No. 7. in the series "Health for All", on the basis of the plan of action approved and accepted by the World Health General Assembly. It also contains an index for Nos 1–7.

After a brief introduction and general comments, Chapter 2 sums up the strategies and plans of action, outlining the general tasks of the member states, the regional committees, the executive board, the World Health Assembly and the directorgeneral. Chapters II to V bearing the titles Developing Health Systems, Promotion and Support, Generating and Mobilizing Resources and Monitoring an Evaluation, set out the tasks outlined in Chapter I in detail to all those concerned (i.e. the member states, the regional committee, etc.). Chapter VI, the longest and the most concrete part of the book, gives a fairly realistic time-table for implementing the global strategy, in a clearly arranged, tabulated form, in chronological order (from May, 1981, to May, 1978) for the Member States, the WHO governing bodies and the WHO Secretariat. On studying the tables one cannot help being impressed by the immense scope and the thoroughness of the activities of WHO displayed in the interest of the implementation of its global strategy. At the end of the book there is an index and a very useful list of references.

L. Cselkó

Education and Training in Occupational Health, Safety and Ergonomics. Technical Report Series 663.

WHO, Geneva 1981. 48 pages. Price: Sw. fr. 3.—

The Joint ILO/WHO Committee on Occupational Health met in Geneva from 2 to 9 March, 1981, to discuss education and training in occupational health, safety and ergonomics. This volume sums up the work achieved by the Assembly, in six parts. Part I gives an outline of the tasks, clearly in the spirit of the objective of WHO "Health for all by the year 2000". Part II on the needs for education and training in occupational health safety and ergonomics, deals with the multiple aspects of these issues and gives a great deal of practical information. Part III discusses the policy in education and training in occupational health and safety, and, after general comments, emphasizes the necessity for purposeful activities at a national, as well as international, level. Part IV, of practical orientation, gives an excellent coverage of the objectives and problems of education and training, discussing first the general objectives, subsequently the objectives by persons who require training. Part V covers various issues of technology, methodology and training programmes. Part VI sums up the recommendations.

In view of its high practical informative value and its clear arrangement, the book excels even among the other members in this series, all of high standard. It closes with a list of 23 references.

L. Cselkó

Primary Prevention of Essential Hypertension. Technical Report Series 686.

WHO, Geneva 1983. 40 pages. Price: Sw. fr. 4.—

The WHO Research Group on the Primary Prevention of Hypertension met in Geneva from 20 to 24 September 1982. An account of the meeting is given in this volume.

In the introduction the basic objectives and motivations are pointed out. In Section 1, two possible approaches to primary prevention of hypertension, i.e. high-risk strategy and mass strategy, are outlined. Section 2 is on the natural history of blood pressure elevations, discussing hypertension in childhood, adolescence and old age, as well as the pathophysiology of early hypertension. Section 3 covers the genetic aspects in consideration of evidence for genetic factors, of the mode of inheritance and of the familial predictors of hypertension. Section 4 of outstanding practical value, deals with the environmental influences in the following order: body-weight, salt and other dietary factors, alcohol, physical activity, psychological social and other influences. Section 5 provides a summary and offers recommendations formulated in 10 points, all highly realistic. In point 7 the need for a flexible adaptation of the health services in the countries of rapidly changing life style to the prevailing requirements is stressed. The volume closes with a list of 130 references.

L. Cselkó

Legislative Action to Combat the World Smoking Epidemic: Roomer, R.

WHO Geneva 1982. 131 pages, 9 tables. Price: Sw. fr. 17.—

The present study, the first WHO publication on the regulatory control of smoking published since the establishment of WHO's Programme on Smoking and Health, 1980, includes 3 sections. It falls into 15 chapters.

Section 1 is subdivided into the following chapters: The smoking epidemics and action by WHO; The role and evolution of legislation to control smoking; types of legislation. An exhaustive and highly interesting analysis is provided by the chapters of Section 2, "Legislation". The most interesting topics include drowing and processing of tobacco, publicity, complete and partial prohibition, health warning and statement of tar and nicotine contents, control of harmful substances in tobacco, restriction of sales to adults, prevention in juveniles and children, restriction of smoking in public places and in the workplace, smoking and healt-education (beginning at school!), fiscal and economic measures, multipurpose statutes in Norway, France, Bulgaria, Finland. Section 3 has two chapters: Challenge to developing countries; Highlights and comments. This is followed by a list of 218 references. In the Appendix we find he legislations of the various countries; the WHO decrees 33 and 35, and the recommendations of the WHO Expert Committee. The practical orientation of the study is one of its essential merits.

L. Cselkó

The Traditional Birth Attendant in Seven Countries: Case Studies in Utilization and Training

Mangay-Maglacas, A., Fizurki, H. Public Health Papers 75. WHO, Geneva 1981. 211 pages with 31 tables, 7 figures and 5 annexes. Price: Sw. fr. 15.—

A world congress on primary health care was held by WHO in Alma Ata in 1978. This volume includes the elaborates of seven countries on the problems of the traditional birth attendant (TBA), centred on the following issues:

1. Ecuador: TBA training programme, supervision, evaluation and follow-up services. 2. Honduras: Administrative arrangements for linking the TBA with the formal health system. 3. Philippines: Development and use of the national registry of traditional birth attendants. 4. Sierra Leone: Practices of untrained TBAs and support for TBA training and utilization. 5. Sri Lanka: Exploring the use of the TBA as a low-cost means for family health. 6. Sudan: Replacing TBAs by village midwives. 7. Thailand: Utilization of TBAs in family planning and maternal and child care.

Any comparison between the reports of the various countries would be futile, since their problems and results are viewed against their own background. The objectivity of information is, however, common to all. It gives particular interest to the elaborates that the facts are considered in their complexity, from multiple angles of approach.

In the closing part the problems of perspectives are summed up and various aspects are pointed out. This part of practical orientation will be of particular use.

L. Cselkó

Prevention of Liver Cancer. Report of a WHO Meeting

Technical Report Series, No. 691, WHO, Geneva 1983. Price: Sw. fr. 3.—

In view of the practical importance of the questions connected with the prevention of liver cancer and of the recent results achieved in this field, a WHO meeting was held on this subject in Geneva, in January, 1983. Though the proportion of hepatitis B-virus (HBV) carriers is relatively low in Hungary, it none the less involves considerable health problems in this country too. In various regions of Africa and Asia earlier infections of the population with HBV attain nearly 100% and the proportion of carriers approximates 15%. The causal relationship between the HBV-carrier state and primary hepatocellular carcinoma has been confirmed by numerous epidemiological studies. On the evidence of a prospective study, conducted in Taivan on the largest scale hitherto known, the HBV-carrier state involves a 223-fold prevalence of hepatoma. According to various estimates, HBV-infections account for 80% of all hepatomas, thus HBV as a carcinogenic risk is second only to smoking. The cause of the high carcinogenecity of HBS-virus may lie in the integration tendency of the virus in the DNA of hepatocytes. Integrated DNS or its essential viral fragments are identifiable in the hepatoma cells in practically all cases of primary hepatocellular carcinoma. Interesting evidence has been furnished by studies of the life-cycles of some animal viruses. In the course of the reproductive cycle a complementary RNA-sequence of viral DNA was identified. This is assumed to be a step, comprising a reverse transcriptase reaction, in the reproduction of the virus. The hepatitis-viruses thus resemble RNA tumour viruses in some of their properties.

The results achieved in HBV-vaccination represent a hallmark in the prevention of HBV-hepatitis. Since it has not been possible to obtain the virus from tissue cultures, carrier plasma has remained the only source of HBsAg. Standardization of the preparations is crucial, for this reason a HBsAg-preparation of known concentration has been developed by WHO. In order to check the preparation for immunogenicity, a mouse strain of known haplotype H2 has been recommended by WHO. In view of their sources, the preparations have to be checked for safety before release, in accordance with existing regulations. Research for safer HBsAg sources s in progress. There are some initial results of cloning methods, but empriments at the use of biological vectors seem still more promising. It is sought to integrate the DNA-sequence of HBsAg into vaccina-virus or into non-pathogenic enteral bacteria.

In regions where HBV-infections are endemic, vertical infections are of major importance. The placenta being impermeable to the virus, the infections occur in the perinatal period. Some reduction in the incidence of perinatal infections has been attained by hepatitis-B immunoglobulin (HBIg). 90% in infants having received HBsAg after birth reveal immune bodies at 6 months, which is a remarkably high proportion at their age. In Japan, 90 to 98% of infants up to the age of 12 months were immune after combined immunisation with HBIg and HBsAg. The efficiency of vaccination was not affected by simultaneous passive immunization. In view of the varying proportions of HBsAg-carriers in the various countries, the preventive measures recommended by WHO also very from country to country. For countries with a high or moderate proportion of carriers a general neonatal combined active + passive immunization, for those with a low proportion, a selective immunization of the groups which are at the highest risk of infection (health workers, etc.) is recommended. In the closing part useful guidelines, are given for the evaluation of the efficiency of immunizations.

This slender volume of 30 pages may be recommended to gastroenterologists, virologists, epidemiologists, neonatologists, oncologists alike. In addition to its practical value, it makes fascinating reading by providing information on various recently disclosed facts about the biology of HBv.

J. FEHÉR

Prevention of Coronary Heart Disease. Report of a WHO Committee.

Technical Report Series No. 678. WHO, Geneva 1982. Price: Sw. fr. 5.—

The WHO Expert Committee on the Prevention of Coronary Hearth Disease held a meeting in Geneva, in December, 1981. The subject-matter on the meeting is covered by this publication of 54 pages.

The mortality figures reflecting the situation in 26 countries are highly instructive. While in a number of industrialized countries the mortality figures in the middle-aged population have shown a considerable decline in the course of the seventies, this is, regrettably, not the case in some other countries including Hungary. It is the prevalence of vascular disease which accounts for the high death-figures. The WHO meeting was convened in order to elaborate a universal preventive strategy taking the generally known risk-factors into account. It is regarded as fundamental to extend the preventive measures to the whole population, starting from childhood, so at to be able to select the high-risk individuals and to subject them to an intensified prevention. In fact, in the industrialized countries the high-risk group accounts for 20% of the population, and nearly 50% of myocardial infarctions occurs in this group. The preventive strategy has proved successful, and even post-infarction prevention has been found rewarding. For the developing countries individual strategies are recommended. In opposition to earlier practice, the objective is not to copy the style of life of the industrialized

countries but rather, without neglecting the positive avenues of development, to maintain those traditional customs which are in keeping with the prevention of coronary disease.

The LDL-cholesterol level is regarded as the most important risk-factor. Its approximation to the total cholesterol level is sufficient for practical purposes. It is desirable to attain average values below 5.30 mmol/l but it is in the region of 4.14 mmol where coronary disease is most uncommon in the population. It is an encouraging fact that in the USA the average cholesterol level has fallen from 6.09 to 5.44 mmol/l since 1950. Preference of proteins of vegetable to those of animal origin, reduction of daily cholesterol intake to less than 300 mg, enhancement of energy utilization, increase in the proportion of complex carbohydrates in nutrition, belong to the main recommendations. On the other hand, therapeutic use of unsaturated fatty acids, regarded by many as efficient, lacks sufficient foundation. Antihypertensive measures play a central part in the prevention of coronary disease. In a given population a lowering of blood pressure has been attained by low-salt diet. The daily intake of salt should be less than 3 g.

Smoking belongs to the essential risk-factors. Nicotine-poor cigarettes carry the same hazards as do the traditional brands. Physical activity has diverse beneficial effects, therefore it is recommended by the Committee beyond its demonstrable benefits. The necessary measures in case of overweight and diabetes are also of major importance. Though among moderate alcohol-consumers the figures of coronary disease are relatively low, yet the demonstrable protective effect of alcohol is too small, while its toxic effects are too grave, to make its consumption recommendable. It is remarkable that, contrary to common belief, the incidence of coronary disease is little affected by the type of personality or by social stress. Therefore, this factor receives no major consideration by the Committee, least it should divert attention from the essential hazards including those of metabolic nature. Use of oral contraceptives in the industrialized countries should be confined to non-smoking, low-risk women. Efficient prevention of coronary disease can be inplemented only at a governmental level. Education of the communities, training of health workers, modification of the general nutritional structure, promotion of physical activity, anti-smoking measures, belong to the main lines of prevention.

In view of the general importance of the question, this publication may be recommended not only to those directly concerned with the subject but to every physician. The reasons for the recommendations are given in a clear, concise form. The essential data are backed by an exhaustive list of references.

J. FEHÉR

Interferon Therapy. Report of a WHO Scientific Group

Technical Report Series 676. WHO, Geneva, 1982. Price: Sw. fr. 3.—

This publication of 28 pages covers the material of the meeting held by the WHO Expert Committee in Geneva, March 1982. The discovery that in cell cultures, under the effect of viral and bacterial infections, chemical stimuli, antiviral protective substances, acting also as immune modulators, are formed, dates some three decades back. These substances have, however, not yet been adopted for current therapeutic use. Three main types of interferon are known: according to the producing cell-type, the alpha, beta and gamma types. The interferons of the two latter types are made up of polypeptide chains of identical sequences, whereas the 12 chains of group alpha are of slightly different sequences. The methods for the production of interferon are no longer confined to buffy-coat, lymphoblast or fibroblast cell-line induction. The DNA recombinant techniques by which interferon of high purity can be obtained, have been gaining increasing practical importance.

Before clinical trials the interferon preparation to be tested has to be checked for safety which, in case of pooled buffy-coat or lymphoblast cultures, poses major problems. In certain experimental systems high interferon doses have displayed considerable neurotoxic and hepatotoxic effects. In human therapy side-effects of this kind are less common and of lesser gravity. In view of the difficulties of comparing interferons from different sources, standard preparations have been developed recently by WHO. It seems promising to induce endogenous interferon by means of chemical inducers although the results of animal experiments are not necessarily applicable to humans.

Sporadic clinical trials which interferon have been in progress for more than a decade. Liberal supplies of standardized preparations enable regular trials, including phase II, to be carried out. Side-effects greatly depend on the purity of the preparation, but even interferon obtained by the recombinant immunosorbent techniques are not entirely pyrogen-free. The side-effects include granulocytopenia, transitory liver function disorders and, at high dose-levels, reversible damage to the nervous system, e.g. convulsions. In a certain proportion of the patients, formation of neutralizing antibodies is demonstrable.

Local or systemic application of interferon has been found of benefit in viral infections of the upper respiratory passages. In herpetic infections, as also in keratitis, it has proved of therapeutic value. Responses of some degree were noted in cytomegalovirus infections, but in the first place after transplantation and in chronic liver disease, particularly if interferon was used in alternation with other antiviral agents. Success is usually transitory, but it has been possible to eradicate the viral genome in chronic hepatitis. The results obtained in juvenile laryngeal papilloma offer the most promising example of the activity of interferon against papilloma viruses. Transitory benefit has been obtained in myeloma and acute leukaemia, partial remissions in 12 out of 40 cases of metastating breast-cancer. Local application of interferon has given favourable results in cases of malignant melanoma, bladder cancer and recurrent glioma. Interferon has been also used with benefit as an adjuvant in osteosarcoma. Its systemic application in melanoma and in non-microcellular pulmonary carcinoma is, however, of no benefit.

In conclusions, the necessity for further trials with standardized preparations has been emphasized by the Committee, and attention is drawn to the still unexplored avenue of approach to the prevention of malignant disease by means of interferon.

J. Fehér

Methods for Cohort Studies in Chronic Airflow Limitation: C. du Florey, S. R. Leeder

WHO, Regional Office for Europe, Copenhagen .European Series, No. 12. Price: Sw. fr. 19. —

This account of the methods for the study of the epidemiology of chronic respiratory disease, issued by the Regional Office of WHO for Europe, has been written by an Australian and a British author. It is divided into five chapters. Chapter 1 describes the various types of epidemiological methods, poiting out their advantages and drawbacks. As the title of the book indicates, the cohort studies, which are actually screening tests on large groups, are best suited for the study of the epidemiology of chronic respiratory disease by linking up cause and effect, even though being more costly than other methods employed for this purpose. In order to make the method efficient, the group under study must be kept intact, therefore its members should be prevented as far as possible from dropping out. Planning as well as implementation of the study must take this point into consideration. Chapter 2 points to the

epidemiological characters of the population samples to be studied and discusses the various difficulties of approach, e.g. spirometry in young children, etc. Information provided on the selection of groups and optimalization of their size, with a clear, concise statistical analysis, will be of particular interest to all those engaged in epidemiologic research. The method based on the use of questionnaires is dealth with in Chapter 3 in close detail. This method has not yet found general acceptance although in some countries it is part of history-taking on admission to hospital. The questionnaire of the Medical Research Council represents a hallmark of historical significance in this respect. It was originally elaborated in England and by not it has been adopted in a modified form all over the world. On the surface, the questionnaire method seems simple but, as pointed out by the authors on the faith of their experience, its efficiency rests on those who ask the questions. Curiously enough, physicians, owing to their directive attitude, are in general less suited for this task than non-medical persons. Anyone to be entrusted with it, receives a thorough training before, in the form of a training course of 8 days, at the end of which he or she may be received or rejected, according to his or her ability. Chapter 4 supplemented by the Appendix, provides exhaustive information on data-processing and statistical analysis. Chapter 5 is on the pertinent documentation methods. In the Appendix an excellent summary of the histamine provocation test is given by one of the authors.

As a source of information meeting the highest standards, the book may be recommended to epidemiologists and pulmonologists alike.

J. FEHÉR

Nongonococcal Urethritis and Other Selected Sexually Transmitted Diseases of Public Health Importance

Technical Report Series 660. WHO Geneva 1981. 142 pages with 14 tables and 5 figures. Price: Sw. fr. 9.—

The volume contains the procedings of a meeting held by the WHO Research Group on nongonorrheal urethritis and other sexually transmitted diseases in Geneva from 20 to 25 November, 1978. It includes 15 chapters, a list of references and an appendix.

By way of introduction, Chapter 1 points to the broadening spectrum of sexually transmitted (s.t.) diseases. In fact, venereology of our days is no longer confined to the five "classic" VD (syphilis, gonorrhoea, chancroid, lymphogranuloma inguinale, donovanosis), but has to deal with numerous other diseases whose sexual transmission had been unknown until recently. The aetiological classification, symptomatology and complications of these diseases are also included in this chapter. Chapters 2 to 7 provide information on s.t. diseases according to the infective agent (Chlamidia trachomatis, Herpes viridae, Hepatitis virus type-B, Ureaplasma urealyticum and Mycoplasma hominis, Haemophilus ducreyi, Cytomegalovirus), in the following order of succession: pathogen, epidemiology, clinical features, laboratory diagnosis, management, prevention. Chapter 8 to 15 describe the following conditions in the same order of succession: trichomoniasis, donovanosis, other important infections (caused by Haemophilus vaginalis, ectoparasites, s.t. enteric agents, group-B streptococcal infections, genital warts), non-gonococcal urethritis, epididymitis, vulvovaginitis, cervicitis and urethritis in women, genital ulcer, pelvic inflammatory diseases. Chapter 16 gives a summary statement of infertility and s.t. diseases, Chapter 17 a summary statement on maternal and infant mortality and s.t. diseases. In Chapter 18 the necessary steps for programme development are outlined. This is followed by a list of 195 references. In Appendices 1–5 recommendations for the therapy of some important s.t. diseases (urethritis, vulvovaginitis and cervicitis, genital ulceration, acute salpingitis), are offered.

L. CSELKÓ

Field Guide to the Detection and Control of Xerophthalmia. Second Eddition. A. Sommer

WHO Genova, 1982. Price: Sw. fr. 10.—

This WHO publication discusses the ophthalmic manifestations of vitamin A deficiency, from hemeralopia to total destruction of the cornea.

This deficiency causing grave consequences occurs at present almost exclusively in the developing countries, particularly in those of Africa, Asia and the West Pacific. Isolated endemic areas exist in the Carribean, Latin American and East-Mediterranean countries. According to recent figures, 5 million children are affected yearly in Asia and 250,000 lose their eyesight.

The book gives ample information on the signs and symptoms, epidemiological aspects, management and prevention of vitamin A deficiency. The 32 colour illustrations serve the diagnosis of xerophthalmia. For examination and diet printed forms are recommended.

MARGIT VARGA

Control of Vitamin-A Deficiency and Xerophthalmia. Report of a Joint WHO UNICEF (USAID) Helen Keller International (IVACG) Meeting, 1980.

Price: Sw. fr. 7.—

This brochure of 70 pages with colour illustrations, instructive tables and graphs comprizes the material of the meeting held by the above organizations is Jakarta between 13 and 16 October, 1980.

A-vitamin deficiency is a grave nutritional disorder causing xerophtalmia and blindness, even marasmus and death in young children.

In the Introduction the advances in research concerned with the biochemistry of vitamin-A and carotine are summed up. After its absorption, vitamin A is stored in the liver in the form of retinol ester. Binding and release result from complex processes, which for the greatest part have been clarified. Vitamin-A deficiency is hardly ever an isolated syndrome; it is practically always associated with protein malnutrition (PEM).

Evaluation of vitamin A deficiency, its biochemistry, diagnostic criteria, ocular and extraocular signs, public health aspects and the methods of supervision receive thorough consideration.

Finally, statistical figures relating to the prevalence of vitamin A deficiency in some regions of Asia, Africa, Latin America and the Caribbean islands are presented. Preventive and therapeutic recommendations are given.

MARGIT VARGA

Luminescence in Biology and Medicine. Editors: L. SZALAY and S. DAMJANOVICH

Publishing House of the Hungarian Academy of Sciences, Budapest, 1983. 421 pages, with 200 figures. Price: 135.— Ft

The book deals with the theory and practice of the classical and new methods of luminescence analysis applied in biology and medicine.

The first three chapters represent a comprehensive introduction to the basis of molecular light absorption and light emission, the methods of luminescence measurement and the application of luminescence in molecular biology.

The fourth chapter discusses the molecular luminescence methods for investigation of cell structure. The topics of fluorescence microscopy and immunofluorescence are treated somewhat too briefly. In spite of this, this chapter will be of aid to researchers working in this field.

This book will be popular among scientists interested in the field of biomedicine and is recommended to all those who apply luminescence analysis in diagnostics.

D. Szabó

WHO Expert Committee on Biological Standardization

Thirty-second Report. Technical Report Series, WHO, Geneva 1983. Price: Sw. fr. 12.—

The objective of the work commenced in 1982 was the elaboration of standards for countries where there are none, or where the standardization of biological components had just started. An essential prerequisite would be the organization of laboratories in each country, which, in possession of international standards, might be able to produce standards for the given country. The desirability of a central elaboration of the working standards is emphasized, but the costs are prohibitive. Recommendations are offered for clinical chemical standards, vitamins and sensitivity tests, vaccines and pyrogens.

In a separate chapter the standards for the various antibiotics, antibodies, antigens, blood preparations, endocrine substances, are defined. The standardization requirements for pertussis, poliomyelitis, Rift Valley fever and measles vaccines are given.

In the 7 chapters of the Annex, the requirements for the above vaccines, furthermore for diphteria toxoid, combined and viral vaccines, as well as for the susceptibility test, are listed under the following headings: manufacturing-national control requirements; requirement for human diploid cells used for virus vaccine production; summary protocol of production and testing.

K. Jobst

WHO Expert Committee on Biological Standardization

Thirty-third report. Technical Report Series, No. 687. WHO, Geneva 1983. 184 pages. Price: Sw. fr. 13.—

Standardization of biological substances involves the interest and the activities of various agencies of WHO, as well as of numerous scientific societies, in various countries. This implies that activities in this sphere are often concurrently employed. For this reason, and also in the interest of the best possible use of the limited material resources, WHO seeks to coordinate these activities. With this purpose an information register of the substances being investigated has been issued. Information on the prevailing situation is given and recommendations are offered under the following headings: test organisms rot antibiotic assay; shake venom and antivenoms; the potency of adsorbed tetanus toxoid; heparin; SPI; thromboplastins; louse-borne human typhus vaccine; oral poliomyelitis vaccine; interferons; pro-

insulins; standardization of monoclonal antibodies, recombinant DNA-technology. In a separate chapter the criteria for the reference substances and guidelines for the elaboration of new international standards are given. The requirements specified here concern antibiotics, antibodies, antigens, blood preparations, endocrine and related preparations, some biologic substances, typhus vaccine, thromboplastin for tests monitoring anticoagulant therapy, polyomyelitis vaccine, antimicrobic susceptibility tests.

In the two appendices of 149 pages, which form the bulk of this small volume, the criteria of standardization are set out in the form of a protocol, and the successive steps of standardization are described in close detail. For interferon standardization and characterization of assays, reference antiserum, neutralization assay, immunoassay, the various standards (HuIFN-alpha, beta, gamma) and their calibration, for the poliomyelitis vaccines manufacturing and national control requirements are considered. Preparation and calibration of thromboplastin are described and the part played by coumarin and factor VII in the assay is pointed out. The book, predominantly of technical character, provides essential, up-to-date aid to all institutions dealing with the standardization of biological substances.

K. JOBST

Laboratory Biosafety Manual

WHO, Geneva 1983. 123 pages. Price: Sw. fr. 14.—

This publication has been intended for heads and supervisors of laboratories, as also for biosafety inspectors, but also contains information for scientists and public authorities responsible for the safety of laboratories in the various countries. The pertaining regulations with regard to organization as well as to handling of pathogens, have varied from country to country until recently. This manual assembles the biosafety recommendation applicable at an international level.

The infective microorganisms are divided into risk groups I–IV, on the basis of individual and community risks. The laboratories are also classed into four risk groups.

Information and recommendation on three main issues are provided under the headings

1. basic standards of laboratory operation, design and equipment;

2. procedures for safe laboratory practice; safety training programmes, safe shipment of specimens, emergency procedures;

3. Selection and use of essential biosafety equipment.

The dangerous laboratory chemicals and the safety equipments are dealt with in a separate chapter. There are 24 instructive illustrations and 6 tables. The volume, of particular use to microbiologists, is well referenced.

K. JOBST

Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes: some Polycyclic Aromatic Hydrocarbons

IARC Scientific Publications No. 49. Lyon 1983. 81 pages. Price: Sw. fr. 18.—

Biomedical research concerned with chemical carcinogens goes hand in hand with the contamination of various materials with carcinogens. Not only the carcasses of laboratory animals, but also the laboratory instruments and the premises of the experiments are contami-

nated. A special programme for the destruction of carcinogen-contaminated laboratory wastes has been elaborated, and is being regularly published by IARC, under the sponsorship of NIH. This volume, the third in the series, concerns the polycyclic aromatic hydrocarbons, i.e. ben(a)-anthracene, benzo(a)pyrene, 7-bromomethylbenz(a)anthracene, dibenz(a,h)anthracene, 7,12--dimethylbenz(a)anthracene, and 3-methyl-chlolanthracene. The methods found suitable for these compounds are presumably also applicable for the elimination of other aromatic carbohydrates and their derivatives.

For the degradation of these products, chemical and physical methods such as oxidation with ozone and hydrogenation have also been applied in addition to biodegradation. These have been summed up in Appendix B. Three methods of an efficiency over 99% are recommended by the working group, i.e. treatment with acid K-permanganate, with concentrated sulphuric acid, with saturated aqueous K-permanganate. The methods, as well as tests for their efficiency, are described in detail. The physical and chemical constants of the chemicals in question are listed in the appendix. The methods described here and in Appendix B are supported by an exhaustive bibliography.

K. JOBST

Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes: Some N-nitrosamines

IARC Scientific Publications No. 43. Lyon 1982. 79 pages. Price: Sw. fr. 20.—

The occurrence of N-nitrosamines and of its precursors in the environment has been on the increase, to begin with foodstuffs, liquor, tobacco. Various chemicals, pesticides, rubber, leather, metal industries provide potential sources. These compounds result from nitrification of mainly secondary, but partly also of primary and tertiary, amines and of quaternary ammonium salts. Though the causal relationship between exposure to N-nitrosamine and human cancer have yet to be conclusively proved, on the ground of experimental evidence the existence of such relationships may be none the less taken into consideration. This requires particular precautions not only in the course of manufacture or preparation, but also of experiments and makes necessary the destruction of various wastes including those from animal sources.

This small volume describes the procedures enabling the chemical carcinogens contaminating laboratory instruments to be decomposed or inactivated 8 compounds, i.e. N-nitrosodimethylamine, N-nitrosodipropylamine, N-nitrosodibutylamine, N-nitrosopiperidine, N-nitrosopyrolidine, N-nitrosomorpholine, and N,N'-dinitrosopiperazine have been tested. The methods described in the book are regarded as suitable for the neutralization of practically all other compounds of this type.

The earlier degradation procedures of questionable value, (Na hypochlorite, chromosulphuric acid, cuprochloride HCl) are critically reviewed. The recommendations are, denitrification with hypochromic acid in glacial acetic acid, photolytic degradation by ultraviolet irradiation, oxidation with K-permanganate in a medium of sulphuric acid, handling with a K-hydroxylated nickel-aluminium alloy, etc. are given. Each procedure is described in detail.

The other (e.g. biological) degradation procedures published in the literature and the chemical and physical constants of the eight compounds listed above have been summed in the Appendix. There is a list of more than 100 references.

The book provides important aid to industrial medicine, laboratories and in the first place to specialists concerned with environmental health.

K. JOBST

10th EUROPEAN CONGRESS OF PERINATAL MEDICINE

Leipzig, German Democratic Republic
August 12-16, 1986

DEAR COLLEAGUES

You are cordially invited to participate in the 10th European Congress of Perinatal Medicine to be held in Leipzig, German Democratic Republic, from August 12th to 16th, 1986.

This Congress is being organized by the "Gesellschaft für Perinatale Medizin der Deutschen Demokratischen Republik" in cooperation with the "European Society of Perinatal Medicine".

The aim of the Scientific Programme is to give participants the opportunity to present the results of their most recent work, to exchange ideas and experiences, to take part in discussions and to establish contacts with foreign colleagues. It is our intention to invite selected specialists to Main lectures, Symposia and Workshops. All scientific problems relevant to the central themes but also Free Papers will be covered during the Congress.

Poster presentations and other scientific expositions will be of great importance and the Organizing Committee is dedicating special attention to them. An exhibition of technical equipments and pharmaceutical products, of medical and belletristic books will be held in conjunction with our Congress.

We do hope to have the opportunity of welcoming you in Leipzig in 1986.

Klaus Jährig
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Hans-Joachim Waraschk
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COMMUNICATIONS

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The official languages of the Congress will be English and German. Simultaneous interpretation will be provide for Main lectures and Symposia.

Accommodation and Travel

All the accommodation facilities will be provided, a number of categories from 5-star-hotels to university hostels has been reserved.

For those participants who wish to take the opportunity of spending a few extra days in the German Democratic Republic, post-congress tours can be arranged.

Social Programme

An extensive social programme is planned for all participants of the Congress and for accompanying persons.

Second Announcement

A second circular containing more detailed information on the Congress, programme, abstract and registrations forms and other instructions will be available in September 1985. To receive a copy, please complete and return the attached form to the Secretariat.

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Applications for training fellowships in 1985—1986 are invited from junior scientists wishing to be trained in those aspects of cancer research related to the Agency's own programme: epidemiology, biostatistics, environmental and viral carcinogenesis and mechanisms of carcinogenesis.

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Fellowship application forms and more detailed information are available from:

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Original articles dealing with clinical and experimental medicine will be accepted with the understanding that they have not been and will not be published elsewhere and are subject to editorial revision.

Form of manuscripts

Two copies of the manuscript typewritten double-spaced with margins at least 4 cm wide should be submitted. Pages should be numbered consecutively. The first page should contain (1) the title of the paper (2) the initials and first name(s) of the author(s), (3) name of the institution where the work was done, (4) name and address of the author to whom correspondence and offprint requests should be addressed — this will appear as a footnote; (5) an abstract not exceeding 250 works which states the purposes of the study, the main findings and principal conclusions. Below the abstract provide 3 to 10 keywords that will assist indexers in cross-indexing the article.

The text of the paper should be divided into sections with the headings: Introduction, Materials (Patients) and Methods, Results, Discussion, References.

Unusual abbreviations should be identified in an alphabetical list typed after the abstract and keywords.

Drugs must be referred to by their WHO code designation (Recommended International Nonproprietary Names); use of proprietary names is unacceptable.

The international system of units (SI) should be used for all measurements.

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These should be cited in the text as numbers in square brackets. The list of references should contain in alphabetical order of the first authors' names the following: authors' last names with initials; for journal articles the title of the paper (lower case), journal title abbreviated according to the style used in Index Medicus, volume number, inclusive page numbers, year of publication in parentheses; for books the title (upper and lower case), publisher, place and date of publication. Only manuscripts accepted for publication may be included in the reference list.

Examples:

1. Stagg, B. H., Temperly, J. M., Wyllie, J. H.: The fate of pentagastrin. Gut **12**, 825—829 (1971)
2. Falkner, F.: Prevention in Childhood of Health Problems and Adult Life. WHO, Geneva 1980.
3. Fishman, A. P.: Dynamics of pulmonary circulation. In: Hamilton, W. F., Dow, P. (eds): Handbook of Physiology. American Physiological Society, Washington 1963, pp. 65—79.

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Each table should be typed on a separate sheet. They should be numbered consecutively with Roman numerals and have a brief specific title. The data presented in the table must be logically and clearly organized and should be self-explanatory. Omit internal horizontal and vertical rules. Cite each table in the text and indicate its approximate place on the margin.

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Figures should be submitted in duplicate. They must be numbered consecutively with arabic numerals. All figures should bear the name of the first author, the figure number and an arrow indicating the top. Cite each figure in the text and indicate its approximate place on the margin. If a figure has been published, acknowledge the original source and submit written permission from the copyright holder to reproduce the material. Figure captions should be submitted typed double-spaced on a separate sheet.

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Acta Medica Hungarica 42 (3—4), pp. 101—108 (1985)

Immunology

EFFECT OF THYMECTOMY IN IMMUNE DISEASES OTHER THAN MYASTHENIA

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In the course of thymectomy of patients with myasthenia gravis, surprising data concerning the recovery or considerable improvement of other immune disorders have been observed. Among these disorders rheumatoid arthritis figured in six instances, immune thrombocytopenia in two cases, polymyositis and psoriasis in one case each. Thymectomy as a powerful immunosuppressive procedure may have a role in the therapeutic management of some immune disorders other than myasthenia gravis.

Keywords: immune disorder, myasthenia gravis, thymectomy, rheumatoid arthritis, immune thrombocytopenia, polymyositis, psoriasis

Introduction

It is well known that myasthenia gravis (MG) may be associated or combined with other diseases such as rheumatic, skin and visceral disorders, haematologic conditions, diseases of the thyroid gland, myopathy, etc. These associations exceeded the expected incidence of these rare diseases, hence the idea has been raised of some possible connection or of a common origin. The observations of these associations did not go beyond registration, especially concerning MG on the one hand, and thyroid disorders and myopathy on the other. The importance of disease-associations has become evident, and the presentation of such cases has become interesting after in MG the possibility of an immune or autoimmune pathomechanism had been suggested, and later successfully proved [5, 19, 32, 41, 51, 54, 55]. In addition to experimental and theoretical data, it was the disease-associations which have provided a considerable argument for the immune pathomechanism of MG, then Simpson [51, 52] stressed the opinion, that MG should be considered a specific manifestation of the general or polysystemic immunopathy localized in the neuro-muscular junction of the motor synapsis.

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It was only natural that attention has been directed in the first line toward those disease-associations in which an immune or autoimmune pathomechanism has been proved or at least suggested. Such diseases are polymyositis, dermatomyositis, systemic lupus erythematosus, rheumatoid arthritis, scleroderma or progressive sclerosis, in a wider sense some of the haemopoetic disorders (e.g. immune or idiopathic thrombocytopenia, pernicious anaemia, haemolytic anaemia), some visceral diseases (e.g. immune glomerulonephritis, Goodpasture syndrome, immune pneumonitis, distress syndrome), and skin disorders such as psoriasis, lichen ruber, candidiasis, some forms of pemphigus and eczema [1, 2, 3, 6, 7, 9, 10, 11, 13, 14, 15, 16, 18, 23, 24, 26, 27, 28, 30, 31, 35, 36, 37, 38, 39, 40, 42, 43, 44, 45, 46, 47, 48, 49, 50, 56, 58, 60, 61, 65, 66, 67].

In the course of 33 years we have observed associations of MG with other disorders in a number of cases. The most important associations could be surveyed on the basis of 750 myasthenic patients observed and followed-up personally by us. The association of MG and thyroid disorders has been discussed in a previous paper [60]. The number and percentage of the diseases associated with MG are shown in Table I where only diseases with certain or suggested immune origin have been included. There was no significant difference concerning associated diseases between the patients having thymoma (15.7%) or thymic hyperplasia (78.2%). The diseases associated with MG had existed prior to thymectomy for MG, there was only a single case where thymectomy was followed by rheumatoid arthritis after six years. A multifold association of thymoma, MG and chronic myelomonocytic leukaemia in an

Table I

Number and percentage of immune diseases associated with myasthenia gravis in 750 cases

Associated diseases	No. of patients	Percentage
Thyroid disease	44	5.86
Rheumatoid arthritis	22	2.93
Polymyositis, dermatomyositis	21	2.8
Systemic lupus erythematosus	7	0.93
Local (discoid) lupus	2	0.27
Diffuse progressive sclerosis	4	0.53
Raynaud phenomenon	3	0.4
Sjögren syndrome	3	0.4
Immune thrombocytopenia	2	0.27
Pernicious anaemia	2	0.27
Psoriasis	4	0.53
Eczematous skin disease	2	0.27
Hodgkin lymphoma	2	0.27
Multiple sclerosis	4	0.53
Myelomonocytic leukaemia	1	0.13
Total	123	16.4

old patient was observed and published elsewhere [23]. In two cases MG was associated with Hodgkin lymphoma [59] and in four instances with multiple sclerosis [57].

In spite of the large literature on the diseases associated with MG and the well-established results of thymectomy in MG, one can hardly find any report on the effect of thymectomy on the associated diseases. In our material 353 patients were treated by thymectomy via median sternotomy. In two female patients who had MG and rheumatoid arthritis, surprising improvement was observed not only in MG but also in rheumatoid arthritis. After this first observation we started to collect data among our myasthenic patients subjected to thymectomy or to large (destructive) dose of thymic X-ray irradiation.

Report of cases

1. *T. E.* a 27 year old woman had developed generalized MG with ocular, bulbar, facial and skeletal symptoms after an airway infection at the age of 18 years. At the onset cholinergic drugs were useful, the course of MG was remittant and benign, but after repeated infections and a pregnancy, exacerbations occurred for 9 years. Rheumatoid arthritis started at the age of 23 years; was progressive in nature and needed constant active therapy. When an active thymus gland (47 g, hyperplasia III) was removed, MG improved rapidly and after a year her rheumatoid arthritis also improved, so that she did not need steroids or other therapy since. During 15 years of postoperative observation MG had to be treated only for short periods, the rheumatoid arthritis was in complete remission.

2. *C. B.* a 22 years old woman. At 19 years of age arthritis developed on her hands, wrists, knees and ankle joints after prolonged exposition to cold. The arthritis caused joint deformities in a short time, during steroid therapy her condition became worse. In the fifth month of penicillamine therapy generalised MG with dominant bulbar and respiratory symptoms developed. MG remained progressive after the cessation of penicillamine therapy for over six months, and she did not tolerate any form of steroid treatment. A very active thymus gland (43 g, hyperplasia of degree III) was removed by sternotomy. In the muscle sample fibre degeneration and regeneration, furthermore lymphotic infiltrations were seen. After the operation both MG and rheumatoid arthritis improved quickly, the patient who up to then unable to move started to walk, her pains ceased. During the next 10 years she needed 120—180 mg pyridostigmine daily, gave life to two healthy children, her rheumatoid arthritis had not to be treated at all.

3. *C. Z.* a 56 years old male patient had been suffering from typical rheumatoid arthritis for 20 years. Finally he was given penicillamine therapy, in the fourth month of which MG developed with dominant bulbar symptoms. MG remained unchanged in the eighth month after cessation of penicillamine therapy, furthermore the patient needed more and more cholinergic drugs. Thymectomy revealed an active gland (36 g, hyperplasia II). After the operation both MG and rheumatoid arthritis improved significantly. During the next 5 years the patient could be kept in remission with low doses of prednisolone and 120—180 mg/day of pyridistigmine.

4. *V. P.* a 14 years old girl. From the age of 9 years she had been treated on account of juvenile rheumatoid arthritis with steroids, azathioprine and penicillamine. At 12 years she had developed ulcerous colitis and two years later MG with skeletal symptoms, then later with bulbar, ocular and respiratory ones. After two years of ceasing penicillamine therapy MG still existed, furthermore it progressed into a crisis-endangered state. At surgery, the thymus gland was large and histologically active (48 g, hyperplasia III). After the operation MG improved gradually, the patient became symptomfree and did not need any cholinergic drug. Rheumatoid arthritis and colitis have also become symptomfree without therapy and in the next five years she has been well.

5. *R. J.* a 35 years old man had been suffering from rheumatoid arthritis for 7 years. In the sixth month of penicillamine therapy generalized and progressive MG developed. After discontinuation of the penicillamine therapy the rheumatoid arthritis became worse and

MG remained unchanged for seven months. Large dosage X-ray irradiation (10×17 cG) was applied to the thymic area. Myasthenic symptoms decreased gradually then ceased, and the rheumatoid arthritis improved considerably. During the next 10 years no treatment was necessary, the patient was able to return to his original job.

6. *V. B.* a 38 years old male patient after 5 years of rheumatoid arthritis had been given penicillamine. After 5 months MG had developed with facial, bulbar, ocular and skeletal symptoms. These decreased after penicillamine therapy had been interrupted, but still continued to exist for over six months. Destructive dosage X-ray treatment on the thymic area resulted in a quick remission of MG and in a gradual improvement of rheumatoid arthritis. The follow-up period has in this case been 5 years, during which only temporary treatment with small doses of steroid or indomethacine was necessary.

7. *P. A.* a 15 years old girl had been suffering from the age of 10 from psoriasis on her whole body and resistant to any therapy, and from temporary acute psoriatic arthritis. Half a year after thymectomy — performed on account of serious generalized MG which endangered the respiratory functions, too, — her psoriasis and arthritis ceased and have not relapsed in the next five years. The thymic gland in this case was hyperactive containing a large number of germinative centres.

8. *K. K.* a 35 years old female had been treated with steroid for over 11 years because of immune thrombocytopenia. At the age of 33 years she had developed MG with progressive generalized symptoms, including respiratory troubles. At thymectomy a very large and active thymus gland was found (46 g, hyperplasia III). After thyemectomy no steroid treatment was necessary during 6 years.

9. *V. P.* a 28 years old female had taken steroids regularly from the age of 19 years on account of immune thrombocytopenia. MG had begun at 26 years with ocular, bulbar and skeletal symptoms. Thymectomy revealed an active gland (27 g, hyperplasia III) with numerous germinative centres. After thymectomy no cholinergic or steroid treatment has been necessary in the last 5 years.

10. *L. L.* a 29 years old male had had MG since the age of 24 years; it progressed into a very serious form with bulbar, skeletal and respiratory symptoms. In addition a progressive polymyositis also developed in his limbs and oesophagus, corroborated by EMG and histologic examination of muscle samples. He had to be treated in IPPR because of respiratory crisis. By sternotomy a very large thymus gland was removed (48 g, hyperplasia III) with numerous germinative centres and lymphoblastic transformation. After the operation, MG improved gradually and symptoms of polymyositis disappeared. No treatment has been necessary in 12 years.

Discussion

The effect of thymectomy on MG was established by Blalock et al. [8] and Keynes [29] more than four decades ago and the benefit of the operation has been proved through forty years. Only the local (ocular) form of MG has been considered an exception, in which thymectomy has remained a matter of debate [22, 25, 68]. In our associated cases thymectomy resulted in recovery in three instances, and in satisfactory remission of myasthenic symptoms in the other seven cases. In five cases MG was caused or provoked by penicillamine therapy [56, 58]. It is an open question whether penicillamine-D would cause a dose-dependent myasthenic reaction which then vanishes after discontinuation of the drug, or it could cause MG which should be considered a true myasthenic disease, or would make manifest a myasthenic process, latent or unknown till the time of penicillamine treatment [58]. This question can only be answered on the basis of collected cases in the future. Our cases seem to suggest the third possibility, as do also some data on the acetylcholine receptor antibodies [17, 21, 53, 62, 63, 64].

In addition to the results achieved in MG by thymectomy, the associated or connected diseases supposingly of immune origin and nature improved in a considerable way and in a significant degree; thus, in four cases of rheumatoid arthritis we saw long-lasting remissions where no treatment was necessary, in two instances the condition improved and small dose of steroid treatment was necessary only temporarily. Total and lasting remission followed thymectomy in two patients with immune thrombocytopenia, the young female patient suffering from resistant psoriasis recovered completely, and a male patient suffering from polymyositis became symptomfree.

The role of the thymus gland in MG has been well known, the data being, nevertheless, in the majority of the cases empiric. The lymphocytes under thymic control can produce antibodies against acetylcholine receptors either inside the thymus or in the blood [33, 34]. These antibodies bound to the postsynaptic (muscle) membrane (acetylcholine receptor site) may cause a partial or total neuro-muscular block with the consequence known as myasthenic fatigue. The thymus can be considered the main regulator organ of immune processes, hence thymectomy is a powerful immunosuppressive procedure. Our observations call attention to the possible role of the thymus in the pathomechanism of other disorders of known or suspected immune (autoimmune) origin and nature. Hence thymectomy may be considered an immunosuppressive treatment in immune diseases other than MG, in spite of the fact that the role of the thymus is even less clarified in these diseases than in MG. In this connection some results achieved by thymectomy in idiopathic polymyositis [4] and multiple sclerosis [12, 20] may also be quoted. It seems to be worth-while to collect further data about the effect of thymectomy on various immune disorders and for this purpose the large MG case material thymectomies seem to be the best solution.

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LYMPHOCYTE MARKERS IN PATIENTS WITH PROGRESSIVE SYSTEMIC SCLEROSIS

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18 patients with progressive systemic sclerosis were investigated. An absolute lymphopenia and a decrease in the number of E-rosettes and early E-rosette forming cells were found. The number of Tγ cells was reduced as compared to the control values. No diminution was found in the number of histamine receptor bearing cells but the number of T lymphocytes capable of recognizing autologous red blood cells was considerably decreased. The number and the ratio of these T cell subpopulations remained relatively stable even after 6 months in the patients with progressive systemic sclerosis. No individual correlation was found between the clinical findings and the ratio of T cell subpopulations.

Keywords: progressive systemic sclerosis (scleroderma), T lymphocyte markers, Tγ cells

Abbreviations: H-binding receptors — histamine binding receptor, PSS — progressive systemic sclerosis, Tγ — T lymphocyte bearing IgG Fc receptors on their surface.

Introduction

Progressive systemic sclerosis (PSS) is characterized by inflammatory, fibrotic and degenerative changes involving the skin and certain internal organs. The majority of investigators found a depressed cell-mediated immunity in patients with PSS. Abnormalities in the distribution of T lymphocyte subtypes [1, 7, 10], the number of E-rosette forming cells [2, 8], leukocyte migration inhibition [9] and antibody dependent cell-mediated cytotoxicity [4] have been described.

Studies on the immunoregulatory helper/suppressor-cytotoxic T cell subpopulation have shown conflicting results [1, 7, 21]. Abnormalities of T cell subsets defined by monoclonal antibodies have recently been described [13, 21]. The majority of observations suggest that the imbalance of the immunoregulatory T cell subpopulations is caused by the diminution of the suppressor T cell subset in patients with PSS [10, 13, 21].

Some of the T lymphocytes bear histamine (H)-binding receptors on their surface. Cells with H-receptors have been found within the OKT8 positive

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population and comprised approximately 50% of the T cell subpopulation having Fc receptors for IgG [14].

Among the very heterogeneous T cells there is a lymphocyte subpopulation capable of recognizing autologous antigenic structures [15, 16]. The lymphocytes forming rosettes with autologous erythrocytes represent a precursor T cell population in which functionally non-committed lymphocytes are also represented [15]. These post-thymic precursor cells may play an important role in immunoregulation. The number of T lymphocytes capable of recognizing autologous red blood cells were found to be reduced in patients with lupus erythematosus [3].

In our study the E-rosette forming cell, T cell subpopulation with Fc receptors for IgG and T cells bearing H-binding receptors were investigated in patients with PSS. Half a year later some patients were reinvestigated so as to study the individual variability in the T cell subsets discussed above.

There is no single diagnostic test for PSS. Abnormalities in the number of T cell subsets may, however, be helpful in understanding the pathogenesis of the disease. This report extends these observations to the investigation of autologous E-rosette forming cells in patients with scleroderma.

Also it is not clear whether immunoregulatory abnormalities represent a relevant factor or an epiphenomenon in the etiology of PSS.

Patients and methods

Eighteen female patients with PSS were investigated. Their mean age was 45 ± 15 years (31 to 64) with a mean history of 9.2 ± 8.3 years. All patients had proximal scleroderma and Raynaud phenomenon, 15 had internal organ manifestations; all fulfilled the preliminary diagnostic criteria for scleroderma [18]. In treatment, D-penicillamine, prednisone and/or nifedipine were applied (Table I).

Table I

Clinical profile of patients with progressive systemic sclerosis (PSS)

PSS with diffuse scleroderma (No.)	18
Age (mean)	45 ± 15
Duration of disease (mean)	9.2 ± 8.3
Number of patients with Raynaud phenomenon	18
Number of patients with	
Raynaud phenomenon	18
Pulmonary involvement	12
Oesophageal dysfunction	8
Cardiac involvement	4
Renal involvement	—
Other manifestations	4
Number of patients receiving	
D-penicillamine (300—1000 mg/day)	5
Prednisone (15 mg/day or less)	4
Nifedipine (30—50 mg/day)	11

Peripheral mononuclear cells were separated on Ficoll-Uromiro gradient. E-rosette forming T cells were determined by the standard method [11]. The number of the early E-rosette forming cells was investigated by the method of Yu et al. [20]. $T\gamma$ cells were demonstrated by IgG-type rabbit antibodies against ox erythrocytes as described by Gupta et al. [6]. H-Binding receptors on T lymphocytes were determined by the method of Saxon [17] and Kedar [12] with slight modifications [19]. The histamine receptor-bearing cells were detected by means of rosette formation with histamine-coated erythrocytes.

The rosette forming capacity of lymphocytes with autologous erythrocytes was determined as described elsewhere [3]. Briefly, 2×10^6 peripheral blood mononuclear cells and 3×10^7 autologous erythrocytes were mixed, centrifuged and before counting incubated at 4 °C over 24 hours.

50 healthy controls were also studied. The results we expressed by means ± S.D.

The differences between groups were evaluated by Student's *t*-test.

Results

Eighteen patients were investigated. The absolute lymphocyte count and the number of E-rosette forming T cells were decreased as compared to the control values (Table II). No diminution was found in the number of histamine receptor bearing cells (Table II). The number and the ratio of $T\gamma$ cells were decreased in patients with PSS as compared to the control values (Table II) and the number of T lymphocytes capable of recognizing autologous red blood cells was also considerably decreased (Table III). The peripheral blood mononuclear cells bearing IgG on their surface were unchanged.

Table II

T cell subsets in patients with PSS

	Patients	Controls	P by *t*-test
Number	18	50	
Absolute lymphocyte count, mm^{-3}	1630±670	2490±675	0.001
E-rosette forming cells, per cent	45±14.5* (780±340)	63±12.8 (850±420)	0.001
Early E-rosette forming cells, per cent	22.5±15.0 (368±288)	34.0±8.4 (850±420)	0.001
T_γ cells, per cent	4.35±3.51* (78±74)	10.4±1.5 (140±40)	0.001
Cells with H-binding receptors, per cent	8.6±5.73 (160±134)	9.6±1.3 (150±30)	NS

* In parentheses: Absolute number of cells in 1 μl blood

Six months later 10 patients with PSS were reinvestigated so as to evaluate again their T lymphocyte subsets. As shown in Table IV, the number of T cell subpopulations remained relatively stable during the 6 month period. No correlation was found between the clinical findings and the ratio of T cell subpopulations.

Table III

Autologous rosette forming T cells in patients with PSS

	Patients	Controls	*t*-test
Number	18	30	
Autologous rosette forming T cells, per cent	9.23±8.50* (166±182)	20.4±3.1 (510±14)	0.001

* In parentheses: Absolute number of cells in 1 μl blood

Table IV

T cell subpopulations reinvestigated 5.7±1.2 months later in patients with PSS

	First investigation	Second investigation
Number	10	
Absolute lymphocyte count, mm^{-3}	1610±840	1554±812
E-rosette forming cells, per cent	48.5±18.9* (828±424)	41.4±21.0 (669±346)
Early E-rosette forming cells, per cent	28.7±14.5 (451±302)	15.7±16.3 (214±171)
T_γ cells, per cent	4.9±43 (85±90)	5.5±5.3 (94±92)
Autologous E-rosette forming cells, per cent	8.2±6.0 (140±100)	11.1±5.5 (198±136)

* In parentheses: Absolute number of cells in 1 μl blood

Discussion

In most studies like in the present one an absolute lymphopenia was observed [2]. Patients with PSS showed a reduction in the number of E-rosette forming T lymphocytes [2, 8], as found also in the present. Recently no significant decrease has been shown when OKT3 monoclonal antibody was used to detect the number of T lymphocytes [21]. The discrepancy was not surprising in view of the fact that two different, partially overlapping T lymphocyte subpopulations were involved in the studies.

The absolute number and the ratio of early E-rosette forming cells exhibited a reduction in the patients with PSS [2], as demonstrated also in the present investigation.

Studies of the helper/suppressor-cytotoxic T cell subpopulations in patients with PSS showed conflicting results. Normal Tγ cells and decreased Tμ cells [1] or increased Tγ cells and decreased Tμ cells [7], and decreased

Tγ cell subpopulations were reported [10]. Recent studies with monoclonal antibodies have shown a T cell imbalance in sclerodermic patients [13, 21]. The elevated ratio of OKT4/OKT8 cells has been attributed to the diminution of OKT8 positive cells [21].

In our study a marked decrease in the number of γ cells was shown. The diminution in the number of E-rosettes, early E-rosette forming cells and γ lymphocytes may have contributed to the depressed cellular immunity in patients with PSS.

Histamine-binding T lymphocytes were found to be present in 50% of the Tγ cells but not in the T cell subpopulation bearing receptors for IgM [14]. In contrast, the histamine-binding T lymphocytes were not decreased in PSS as compared to the control patients.

In our study a reduction in the autologous E-rosette-forming cells was demonstrated in sclerodermic patients. To our knowledge this diminution of autologous E-rosette forming cells has not been described previously. The reduction in the post-thymic precursor cells may provide one of the factors for the altered immunoregulation in patients with scleroderma.

Half a year later 10 patients with PSS were reinvestigated. The ratio and the number of E-rosette, early E-rosette forming cells, Tγ lymphocytes and autologous E-rosette forming cells showed a relative stability during the 6 month period.

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DEMONSTRATION OF THE EFFICIENCY OF SPECIFIC MUCOUS MEMBRANE DEFENSE BY FACTOR ANALYSIS

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Gastric juice and bile fractions A, B, C of 143 patients, vaginal secretion and cervical mucus of further 257 patients, and the sera of all patients, were studied for IgG, IgA, IgM, complement C3, albumin and transferrin, together with acidity tests and microbiological study. The data thus obtained, nearly 10 000 in number, were subjected to automated (factor) analysis. This revealed certain concealed relationships which has been superimposed by the manifestations: the specific defense of the mucous membranes resulting from the activity of the secretory immune system takes effect only under special conditions. The local secretory immune factors, together with the effects connected with acidity and proteolytic activity, are deployed only in the stomach and duodenum at the first level of mucous membrane defense. For the defense of the genital mucous membranes — in the period of sexual activity and reproduction — the transudative factors of the serum resulting from hormonal activity are responsible, and only if these factors are affected does the specific defense mechanism of the mucous membranes take action.

Keywords: secretory immune system, acidity, gastroduodenal juice, vaginal secretion, cervical mucus, factor analysis

Introduction

Bacteria, fungi and viruses are direct constituents of our intrinsic and extrinsic environment. The antigenic environment of the mucous membranes differs, therefore, widely from that of the general immune system. For instance, the alimentary tract is an abundant source of live and inanimated antigens: the mucous membranes are in constant communication with many thousands of various substances acting as immune stimulants or immune modulators.

The antigens may pass across the mucous membranes atively or passively and gain access to the immunocompetent lymphocytes which they stimulate, or controversely, in which they induce tolerance as a result of complex regulatory mechanisms [13].

Studies of the microbial colonization of the mucous membrane, of the regulation of the microflora and of the relationships between these processes, have been attracting increasing interest. The organism has multiple defense mechanisms against antigenic invasion of the integument [3, 5, 7, 14].

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The discovery of the specific defense system (secretory immune system) allowed to confirm various hitherto unexplained facts, e.g. nasal or enteral immunization. This gave food to the tendency of incriminating specific, rather than the well-known non-specific, factors for the manifestations of certaiu phenomena, and may have accounted for confusing data relating to the body-fluids, as illustrated by the findings concerning the genital secretions [15]: there are findings confirming the predominance of IgG on the one hand [15], and of IgA [16], on the other. While, according to some authors the gastric juice contains no immunoglobulins [14], according to others it does contain proteins of a highly variable pattern [6].

Research in the last years has been lending increasing support to the significance of the non-specific defense mechanisms of the body [1, 5, 7]. The necessity for considering not only the specific, but also the non-specific immune factors in studies of the defensive activity of the mucous membranes was, therefore, self-evident.

For the explanation of the above relationships, the literature has little to offer. This is because no major progress in research concerning the immunological barrier of the mucous membranes has been achieved until the last decades.

We have, therefore, undertaken a complex study of the defense mechanisms of the mucous membranes, limiting the demonstration of the phenomena in question to two mucous membrane regions of dermatological relevance, which give fairly easy access to the clinician and which are the sites of a high antigenic activity even under physiological conditions. On these grounds we have selected two models for the study of the essential factors of mucous membrane defense, samples of duodenal juice collected by gastroduodenal tube, and vaginal secretions. There is a particular factor interfering with the defense activity of the mucous membrane barrier of either region, i.e. the reservoir character of the stomach and the cavity-character of the vagina. Moreover, the factors involved in mucous membrane defense operate at a high intensity even under normal, and still more under abnormal, conditions.

The material for demonstration of mucous membrane activity was provided by gastroduodenal juice samples obtained from selected patients who had some indications of enteral dysbacteria in their history, and vaginal secretions obtained from leukorrhoeic patients of Harkány Hospital.

Our former observations in a number of cases belonging to the same patients material have been reported earlier [10]. It has been also demonstrated that though the protein concentration in the secretions is lower in the stimulated than in the resting state, protein transflux (volume $\times$ concentration) quantifiable from the samples is practically identical [11]. We found it, therefore, sufficient from the point of view of comparability to take into account the protein concentrations measured in the samples.

The study of the specific and non-specific factors has provided us with a vast body of data, evaluation of which required an automated technique. The conventional biostatistical methods were supplemented by the analysis of background factors. Factor analysis allowed to disclose the responsible background variables factors of a multivariety system [12]. The basic idea was that the majority of our observations and correlation studies registered merely manifestations of the phenomena, without being able to disclose the essential background variables. On the other hand, the multivariate system, in view of its collinearity, allowed to describe a set of factors in terms of one or more single common factor. Therefore, many variables can be replaced by very few factors which include all these variables. For the number of these factors two criteria have to be considered, first that the number of factors must be sufficient for matching the factor model to the set of variables, and it must also be suitable for proper interpretation. Recognition of the variables being at the background of the cluster of phenomena, definition of their number and their numerical expression offer the most rewarding line of research.

Material and methods

Duodenal juice samples of 143 patients with skin diseases, 75 males and 68 females, of a mean of age 40.3 years, were studied. Only the data of those cases were considered from which 4 samples (gastric juice, bile fractions A, B, C) could be obtained. Thus a total of 572 samples was studied. Samples of vaginal secretion and of cervical mucus were studied in 257 gynaecological patients of mean age 34.4 years, 78% being aged between 21—40 years. A total of 514 samples was studied. The sera were studied in all of the cases.

The bodyfluids were subjected to microbiological (bacteriological, mycological, parasitological) studies, and their pH was also estimated.

Immunological methods: IgG, IgA, IgM, complement C3, transferrin and albumin according to Laurell [8], and Mancini et al. [9], respectively. On reproduction of the assays the variations remained within 10%.

Biostatistical methods. The objective of automated processing was to evaluate the data of measurements, nearly 10 000 in number. The statistical sources were provided by the quantifiable informative data of the patients (e.g. age, sex, pH, etc.) and by the results of the measurements. The data were collected on code-sheets and transferred to perforated bands, subsequently to magnetic plates. It was after formulation of the theoretical concept that the basic information required by the studies was selected. The data were evaluated by computer Type R22 (M. Pollack University of Technical Sciences, Pécs), in accordance with program BMDP (1978, Health Science Computing Facility, University of California, Los Angeles), P 4 M chapter Factor Analysis.

The evaluating program furnished the following results: 1: mean values with S.D. of all parameters, the minimum and maximum values of measurements; 2: the degree of correlation between the paired parameters; 3: results of the F-test and two-sample *t*-test for all parameters in two arbitrary groups including the degree of freedom and the computed *t*-values; 4: factor analysis. The present report deals with the results of factor analysis. The data thus outlined is permit to disclose the correlations between the original variables and factors. Factor loading expresses the correlations between the variables and the factors. Therefore, the primary task of factor analysis was to determine factor loading. According to the concept of factor analysis, the individual variables are occupied, more accurately shaped, by the various factors, the degree of which is reflected by the factor load. The variance of each factor in the whole set of variations is expressed in the cumulative proportion of total variance in per cents. On theoretical grounds there is no correlation between the factors, since so long as they are correlated, they have a common factor (up to the limit of correlation), and the system is still open to further "factorization".

Table I

Results of factor analysis

I. Gastroduodenal juice n = 143		II. Vaginal secretion n = 257	
common factor*	factor loading**	common factor*	factor loading**
I. *local IgA and IgG factor*		I. *vaginal transudable factor*	
A-IgA	0.844	F-IgG	0.790
B-IgA	0.806	F-transferrin	0.718
A-IgG	0.800	F-C3	0.689
C-IgA	0.782	F-albumin	0.697
C-IgG	0.743	F-IgM	0.608
B-IgG	0.723	F-IgA	0.581
G-IgA	0.711		
G-acidity	0.561		
G-IgG	0.554		
G-microbial content	0.548		
II. *complement factor*		II. *cervical transudable factor*	
C-C3	0.922	C-transferrin	0.839
B-C3	0.921	C-albumin	0.765
A-C3	0.898	C-C3	0.745
III. *albumin factor*		III. *serum factor*	
B-albumin	0.845	S-IgG	0.773
A-albumin	0.723	S-IgA	0.740
C-albumin	0.689	S-IgM	0.617
IV. *transudable factor*		IV. *local IgA factor*	
G-C3	0.880	C-IgA	0.680
G-IgM	0.643		
G-IgG	0.554		
V. *serum factor*		V. *pH and age factor*	
S-IgA	0.594	F-pH	0.793
S-IgG	0.572	age	0.604
S-C3	0.502		

Commentary:

1. type: G: gastric juice
A: A bile
B: B bile
C: C bile
S: serum

1. type: F: vaginal secretion
C: cervical mucus
S: serum

* *common factor:* unit influencing a set of observed variables
** *factor loading:* degree of loading of the common factor by the variable

Results

The difference in the results of factor analysis, as far as the order of succession of the protective factors of the two mucous membrane surface (gastroduodenal, and vaginal) under study was concerned, may imply that the defense activity of the immunoglobulins produced locally in the mucous membranes asserts itself only under particular conditions. Table I represents the common factor derived from the computerized data of the two groups, and the factor loadings by the observed variables included in the individual factors. Table II shows the cumulative proportion of total variance of 5 factors in each of the two groups classified on the basis of the respective data.

Table II

Interpretation of the results of factor analysis

I. Gastroduodenal juice n = 143		II. Vaginal secretion n = 257	
common factor	%*	common factor	%*
I. *local IgA and IgG factor*	26.7	I. *vaginal transudable factor*	30.5
II. *complement factor*	38.0	II. *cervical transudable factor*	41.2
III. *albumin factor*	46.2	III. *serum factor*	49.2
IV. *transudable factor*	54.1	IV. *local IgA factor*	56.3
V. *serum factor*	61.4	V. *pH and age factor*	62.7

Commentary:

*%: cumulative proportion of total variance

In the *gastroduodenal* samples the first four factors were provided by local factors demonstrable in the bodyfluids. Factor V was provided by serum factors.

The elements of Factor I were formed by IgA and IgG with a high factor loading. In addition, non-specific factors (pH, microbes) were also present. The factor by itself was responsible for the manifestation of 26.7% of the phenomena.

Factor II included the factors of complement C3 of the duodenal juice, with a very high factor loading. The first two factors together accounted for the manifestation of 38% of the phenomena.

Factor III comprized the albumin factors measured in the gastroduodenal juice. The three factors together accounted for 46.2% of the manifestations.

Factor IV was constituted by complement C3, IgM and IgG demonstrable in the gastric juice. The four factors together accounted for 54.1% of the manifestations.

Factor V was formed by protein components IgA, IgG and complement C3 measured in the serum. The five factors together accounted for nearly two third (61.2%) of the manifestations.

In the *female genital tract* the first three factors were provided by transudable proteins originating from the serum. Factor IV included IgA secreted by the cervical mucosa.

The elements of Factor I were prevalently transudable proteins demonstrable in vaginal secretion, i.e. IgG, transferrin, complement C3, albumin, IgM and IgA. The factor by itself accounted for 30.5% of the manifestations.

Factor II was also formed by transudable proteins, transferrin, albumin, complement C3 and IgM, with a high factor loading. The first two factors were responsible for 41.2% of the phenomena.

Factor III was formed by IgG, IgA and IgM measured in serum. The three factors together accounted for 49.2% of the manifestations.

IgA measured in the cervical secretion was only fourth in order, forming Factor IV. The four factors together accounted for 56.3% of the manifestations.

Factor V comprized the pH and age. The five factors together accounted for nearly two thirds (62.7%) of the manifestations.

Discussion

The five gastroduodenal factors, as well as the five vaginal factors, accounted for two thirds of the manifestations of the respective phenomena.

The antibodies released as a result of specific activity might have been expected to play a primary part in mucosal defense. The results of factor analysis, as far as the order of succession of the involvement of the defense factors was concerned, however, not identical for the two mucosal surfaces under study. This seemed to suggest that the specific factors of mucosal defense manifest themselves only under particular conditions.

The first line of gastroduodenal mucosal defense is formed, side by side with local immunoglobulins (specific factors), by non-specific factors, i.e. pH and proteolysis (Factor I). As it is known, proteolysis of antigens and of antibodies of protein character proceeds in the stomach and duodenum also under physiological conditions (normacidity, proteolytic activity). In addition, gastric HCl, by virtue of its coagulants, bactericidial and bacteriostatic activ-

ities, may play a major part in the elimination of antigens [1, 5, 7]. It seems, therefore, obvious that Factor I includes, side by side with the local immunoglobulins IgA and IgG, the chemical reaction and the antimicrobial factors. The possibility of a complement activation by a classic or an alternative mechanism alike [12] may account for the finding that the elements of Factor II are complement factors demonstrable in the bodyfluids. These are produced by the macrophages (prevalently in the liver), thus attaining the mucosal surfaces as a result of transudation, i.e. through vascular pathways.

Factor III was formed by albumin components demonstrated in the gastric juice. Albumin is produced in the liver, therefore it is only via the circulation (as a result of transudation) that it attains the mucosal surfaces. The presence of the complement (II)- and of the albumin (III)-factors confirm, in addition, that in the course of an inflammatory process elicited by some local antigenic stimulus, general immune reactions (related to the circulating antibodies) may also take part in the elimination of antigens. This implies that the general immune response also represents a factor of immune defense. Factor IV was also formed by transudable proteins: complement C3, IgM and IgG of the duodenal juice. Factor V comprized factors IgA, IgG and complement C3 originating from serum. This finding is consistent with the possibility of a vascular origin (blood or lymph) of certain proteins, as pointed out above.

In the defense mechanisms of the *female genital system* the transudative factors and those originating from the serum predominate; in their background hormonal factors may be assumed. As it is known, the sparse immunocytes of the vaginal lamina propria are unable produce immunoglobulins in any significant quantity, moreover, the non-keratinizing squamous epithel cells fail to produce the glycoproteins of transport function, indispensable for the formation of secretory immunoglobulins [3, 4, 14]. The conditions are thus adverse to the production of secretory immunoglobulins. The protein components identified in the vaginal secretion have none the less been shown by factor analysis to belong, despite the anatomic situation, to Factor I. This must be attributed to vascular, rather than to local, specific factors, resulting in all probability from transudative processes attributable to vascular reactions. In the period of sexual maturity exemplified by the present cases the vascular reactions are subject to hormonal regulations. The findings that the cervical tissues by its anatomic structure is capable to produce immunoglobulins in substantial amounts and that, as shown by factor analysis, Factor II also includes transudable proteins, transferrin, albumin, complement C3, IgM (majority of these proteins formed in the liver), are consistent with the role of vascular reactions resulting from hormonal regulatory mechanisms.

The role and significance of the first two transudative factors is highlighted by the finding that the constituents of Factor III originate from serum.

IgA of local cervical production belongs to Factor IV. Factor V includes, in addition to the factors related to the pH, the element of age. The chemical reaction is primarily age-related (hormonal activity). The age-factor may reflect menopausal changes.

Factor analysis has thus allowed to disclose certain relationships which had been concealed by the manifestations. These may be summed up as follows. The local specific immune factors take effect, together with factors of acidity and proteolysis, only in the stomach and duodenum, thus forming the first line of defense. The defense of the genital mucous membranes, at least in the period of reproduction, rests primarily on vascular factors resulting from transudative processes involving a hormonal activity. Though here too, the specific mucous membrane defense is significant (local factor IgA), its stimulation into action requires particular circumstances, e.g. a malignant process.

The results of factor analysis have thrown some light on certain new aspects of the complex defense mechanism of the mucous membranes. It emerges from the facts disclosed by the study that their defense, similarly to the hormonal regulations, results from the interplay of various specific and non-specific factors and mechanisms operating at different levels.

In conclusion, the function of the secretory immune system may be regarded as an activity in its own right serving the integrity of the body. Its potential compensatory role in various chronic processes adds to its significance.

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IgE LEVEL IN SOME DERMATOLOGICAL DISEASES

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The total IgE level was measured by radioimmunosorbent test (Phadebas RIST, Pharmacia) in blood samples of 454 dermatological patients and 26 blood donors aged 14—70 years. The results showed different IgE values in the same groups of diseases. Elevated serum IgE values were observed in atopic dermatitis, drug allergy, discoid lupus erythematosus and chronic dermatomycosis. In drug allergy the average non-specific IgE level of patients reacting with immediate reaction was higher than the normal value.

Tested individually with Penicilloyl G RAST no strong correlation could be demonstrated between the quantity of specific and non-specific IgE.

Keywords: Serum IgE,— RIST — Dermatological diseases.

Introduction

The IgE molecule is similar in structure to the other immunoglobulins but has some different characteristics as well. It has an unusual affinity to basophils and mast cells, that is why it is deposited in certain tissues ("shock organs"). The serum IgE level is considerably lower than that of other immunoglobulins and shows a high variability according to age and genetic constitution. IgE, discovered by Ishizaka [5] and Juhlin [6] played a prominent role in clarifying its structural and biochemical properties. In view of its a low serum level isotope methods are used for detecting IgE.

In a previous paper we have shown that the serum IgE level may be elevated in some skin disorders and in immediate type drug hypersensitivity [9, 10, 11, 12]. In the present paper we report on our work performed in the period 1974—1982.

Material and methods

The serum IgE level was determined in 480 subjects: 454 had various skin disorders and 26 were healthy blood donors. They ranged in age between 14 and 70 years; males and females were in about equal number.

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The radioimmunosorbent test (RIST) (Phadebas RIST IgE kit) was used to detect IgE as described previously [9]. Values were given in kU/l. As the serum IgE levels do not follow the Gaussian distribution and show a high variance, bot the arithmetical and geometrical means had to be known since [17] only on the basis of the latter could the significance be calculated as it is seen Table I. To compare the values of patients and controls, Student's *t*-test was performed.

Results

The arithmetical and geometrical means of the total IgE levels measured in patients and controls and the statistical calculations are shown in Table I.

Table I

Serum IgE values in skin diseases

Diagnosis	N	kU/l $\bar{X} \pm SE$	GEOM $\bar{X}$	P
Drug allergy	130	1770.84±149.84	689.36	* * *
Lupus erythematosus disc.	63	1385.71±202.35	929.81	* * *
Photodermatosis	47	1566.38±308.14	933.83	* * *
Chronic dermatomycosis	73	1189.18±152.62	662.36	* * *
Contact eczema	18	590.00± 84.57	464.76	*
Atopic dermatitis	44	2850.22±374.92	1765.85	* * *
Chronic urticaria	14	637.86±237.02	367.45	N S
Other dermatoses	65	1800.62±137.02	485.20	* * *
Control	26	315.00± 38.18	265.66	

It can be seen that almost in every skin disorder the serum IgE level was elevated. Its mean value revealed big differences among individuals in every disease group. The individual and mean values were extremely high in atopic dermatitis and significantly above normal in drug allergy, discoid lupus erythematosus, photodermatoses and chronic mycoses. The values were lowest in contact eczema and chronic urticaria. The values in the "other dermatoses" group were heterogeneous.

The individuals examined could be divided into three groups according to their total IgE level.

Group I. $<$ 400 kU/l
Group II. 400—2000 kU/l
Group III. $>$ 2000 kU/l

The percentage of these groups is demonstrated in Fig. 1. It can be seen that the distribution of the values was different in the various groups. A medium level was most frequent in drug allergy, lupus erythematosus discoides, photodermatosis, chronic urticaria and other disorders. In contact eczema the IgE level was found mostly low or medium while in atopic dermatitis, medium or high.

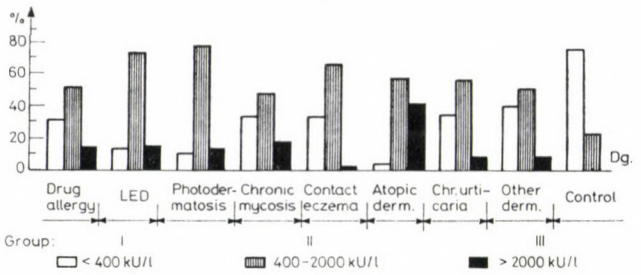

Fig. 1. **Percentage distribution of IgE levels**

Values under 400 units were measured in several cases of contact eczema, while only a few in atopic dermatitis. An elevated level occurred in every patient group, but in no subject in the control group where the highest IgE value was 820 kU/l.

Discussion

Barsoum and Kuwert [2] found high IgE levels in the healthy population and his work drew our attention to the importance of dividing the values into three groups. The actually measured serum total IgE level is influenced by age, genetic and environmental factors. The antigen-specific and total IgE level is regulated by different genes and the genetically determined IgE levels are modified by environmental (allergen exposition) and internal (effector and suppressor) factors. The actual IgE level is the resultant of these factors, the variability of which has been proved [8].

Elevated IgE levels were determined in both healthy individuals and those suffering from skin diseases. The levels were highest in hyper IgE syndrome and in atopic diseases [3, 4, 5, 6, 14, 16]. Parallel to IgE determination we also detected the IgG, IgA and IgM levels in every case using Mancini's radial immunodiffusion method The mean values of these globulins were within the normal range in every case.

In certain disease groups we determined T-cell count by the E-rosette method [11, 13]. Except in contact dermatitis, the E-rosette count was lower in every disease group of negative correlation was found between the average IgE level and the E-rosette forming cell count.

Specific IgE detection by RAST was performed only in penicillin sensitive patients and we could not find any close correlation between the specific and total IgE values [12].

In the light of the present investigation and data in the literature an elevated IgE level cannot be considered pathognomonic.

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T LYMPHOCYTE SUBPOPULATIONS IN PROGRESSIVE SYSTEMIC SCLEROSIS DEFINED BY MONOCLONAL ANTIBODIES

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Twenty two patients with progressive systemic sclerosis were studied by monoclonal antibodies to detect OKT4 and OKT8 positive cells. The absolute number of OKT4 and OKT8 cells was not altered as compared to control values. The ratio of T4/T8 cells slightly increased without statistical significance. The number of E-rosette forming T-cells was reduced in patients with progressive systemic sclerosis. Six months later 11 patients were reinvestigated, and similar results were obtained for the T-cell subsets and the ratio of T4/T8 positive cells. Individual data showed marked variability in the two periods of investigation without however, any correlation to the clinical findings. The sclerodermic patients had a long disease duration and showed no immunoregulatory T-cell imbalance.

Keywords: Progressive systemic sclerosis, monoclonal antibody, helper cell, suppressor cell

Abbreviations: PSS — progressive systemic sclerosis
T_γ cells — IgG Fc receptor bearing T lymphocytes
T_μ cells — IgM Fc receptor bearing T lymphocytes

Introduction

Progressive systemic sclerosis (PSS) is characterized by fibrotic, degenerative and inflammatory changes involving the skin and certain internal organs. The cause of PSS is unknown but a dysfunction of the immune system may have been involved. There is no characteristic laboratory test for PSS. The imbalance in the ratio of helper/suppressor cytotoxic T-cell subsets may have a pathogenetic importance. The immunoregulatory OKT4/OKT8 T-cell subsets were described previously in PSS [1, 4, 5, 7], but these studies gave conflicting results. In a previous study we found a significant decrease in the ratio of $T\gamma$ cells in PSS. Abnormalities of T-cell subpopulations defined by monoclonal antibodies have recently been described [3, 8, 12].

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Our study was designed to determine the OKT4 and OKT8 positive T-cell subpopulations in patients with PSS. One úalf year later túe patients were reinvestigated, to study the individual variability in the number of T-cell subsets.

Patients and methods

Twenty two female patients with PSS and 10 volunteers were studied. Their mean age was 43.7 ± 13.7 (31 to 61) years. The mean disease duration was 9.1 ± 8.3 years. All patients were in a stable condition, 19 had manifestation(s) of internal organ(s). Pulmonary involvement was detected in 15, oesophageal dysfunction in 12, cardiac involvement in 6 cases. Manifestation of other internal organs was found in 4 patients. One patient had renal disease. All patients fulfilled the preliminary diagnostic criteria scleroderma (10). Basic therapy included nifedipin in 15 patients, D-penicillamine in 7 and prednisone in 2 cases. No drugs were administered 24 hours before the investigation. Eleven patients were reinvestigated 6 month later. E-rosette forming T-cells form peripheral blood mononuclear cells were determined by the standard method.

T-cell subsets were investigated with OKT4 and OKT8 monoclonal antibodies (Ortho Pharmaceutical Inc., Raritan, N. J.) by indirect immunofluorescence technique as described previously [11]. The results are expressed as mean ± S.D.

Results

In the 22 patients with PSS the absolute lymphocyte count and the number of E-rosette forming T-cells were decreased as compared to the controls (Table I).

Table I

T-cell subsets in patients with PSS

	Absolute lymphocyte count (mm^{-3})	E-rosette forming cells, per cent	OKT4 per cent	OKT8 per cent	T4/T8 ratio
Patients (n = 22)	1630±670*	46±14.5* (780±340)**	38.7±12.7 (700±340)	14.9±7.7 (400±300)	2.9±1.4
Controls (n = 10)	2190±520	70±15.1 (1530±330)	44.6±10.7 (960±260)	19.5±4.7 (410±70)	2.4±0.5

* $p < 0.001$ by Student's t test
** the absolute number of cells (mean ± S.D.) in 1 μl blood are in parentheses

The ratio of OKT4/OKT8 cells was slightly increased but this augmentation was not significant statistically (Table I). In accordance with other authors [8], we found that the patient group exhibited a much wider scatter of data than did the control values for T4 and T8 markers.

No alterations were found in the number of OKT4 and OKT8 positive cells as compared to the control values (Table I).

Six months later 11 patients were reinvestigated to determine their T-cell subsets. As shown in Table II, no changes in the ratio and the number of T-cell subpopulations were found as compared to the control values.

Table II

T-cell subsets in patients with PSS reinvestigated 5.7 ± 1.2 months later

Investigation	Absolute lymphocyte count (mm^{-3})	E-rosette forming cells, per cent	OKT4 per cent	OKT8 per cent	T4/T8 ratio
First	1600±800	51.6±20.2 (890±550)*	36.7±16.9 (670±399)	13.2±8.0 (230±160)	2.95±1.7
Second	1550±770	43.6±21.2 (700±340)	31.5±16.0 (500±220)	15.2±8.5 (254±155)	2.33±1.7

* the absolute number of cells (mean ± S.D.) in 1 μl blood are in parentheses

11 patients with PSS were investigated. Their age was 43.1 ± 16.9 years. The mean duration of the disease was 10.0 ± 9.1 years.

The number of E-rosette forming T-cells remained stable during the 6 month period but the individual data of OKT4 and OKT8 subsets showed a marked variability at the two different periods of investigation. No individual correlation was found between the clinical findings and the ratio of T-cell subpopulations.

Discussion

In most studies and in the present one an absolute lymphopenia has been found in PSS [2, 4, 6] together with a reduction in E-rosette forming T lymphocytes [2, 4, 5, 12]. The latter deficiency was shown also in our previous study [4], but no significant decrease was shown when OKT3 monoclonal antibody was used to detect the number of T-cells [8, 12].

Studies reporting on the cytotoxic-suppressor/helper T-cell subpopulations in patients with PSS showed conflicting results. Normal T_γ with decreased T_μ cells [1], increased T_γ cells with decreased T_μ cells [5] and decreased T_γ cell populations were reported [7]. Our previous study also showed a significant decrease in the ratio of T_γ cells in PSS [4].

Recent studies have shown a T-cell imbalance in PSS determined by monoclonal antibodies [3, 8, 12]. The elevated ratio of OKT4/OKT8 cells has been attributed to the diminution in the number of OKT8 cells, which remained stable during a 1 year period [12]. No correlation was found between the elevated ratio of OKT4/OKT8 cells and the in vitro suppressor function of these cells [12]. In another report an increased helper function in vitro was demonstrated

in patients wiht PSS [9]. In one of the studies the majority of the patients with an elevated ratio of OKT4/OKT8 cells were found to be generally younger and to have a short disease duration [8].

In the present study, sclerodermic patients with long disease duration had no immunoregulary T-cell imbalance. The 11 patients reinvestigated one half year later showed the same result with marked variability of the number and ratio of T4/T8 cells. The decreased number of E-rosette forming cells remained stable during the time of investigation. Patients with an elevated ratio of OKT4/OKT8 cells were not clinically distinguishable from the other patients.

The discrepancies in the finding of T-cell subsets could be explained by differences in the duration of the disease, the small number of patients investigated and the wide geographic variations in the populations studied.

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Endocrinology

EFFECT OF DOMPERIDONE ON SERUM TSH AND GROWTH HORMONE IN THYROID PATIENTS

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In euthyroid female patients, the release of TSH from the pituitary increases in response to domperidone, a dopaminergic-receptor blocking agent of peripheral action. The rate of increase varies with the functional state of the thyroid, as confirmed by results obtained in cases of euthyroidism, primary hypothyroidism, subclinical hypothyroidism and subclinical hyperthyroidism. Observations suggest that the more vigorous feedback-mechanism modulates the dopaminergic regulation of TSH-secretion at a high degree of sensitivity. Stimulation with l-dopa intensifies the release of growth hormone from the pituitary which is, however, of lesser degree in hyperthyroid or hypothyroid than in euthyroid individuals. The GH-response to l-dopa is enhanced by adminsitration of propranolol, but the maximum serum GH levels in response to stimulation with l-dopa are significantly lower in hyperthyroid than in euthyroid individuals. Administration of domperidone leaves the serum GH levels unaffected in euthyroid and hyperthyroid subjects, but causes a significant increase in a number of patients with primary hypothyroidism.

The results suggest that the dopaminergic system plays a part in the regulation of TSH and GH secretion, asserting itself partly as a direct effect on the pituitary, and that the dopaminergic regulation may be affected by thyroid dysfunction.

Keywords: Serum TSH level, growth hormone level, domperidone, thyroid disease.

Introduction

The therapeutic use of dopamine-receptor blocking agents has been gaining ground in recent years. These drugs are mainly used for the control of nausea or vomiting and in disorders of intestinal motility. They also act on the central nervous system, primarily as stimulants of prolactin secretion. A particular group of these drugs (metoclopramide, sulpiride) is marked by a central nervous and hypophyseal activity [, 32], whereas those of the other group (domperidone) fail to pass the blood-brain-barrier and exert their action at the periphery on the eminentia mediana and directly on the pituitary [8, 32].

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The aim of the present study was to examine the secretory responses of TSH and of growth hormone (GH) to stimulation with domperidone in various states of thyroid function.

Materials and methods

Female patients aged 25—50 years, with euthyroidism, untreated Graves' disease, subclinical hyperthyroidism, subclinical and manifest, primary hypothyroidism were studied. Hyperthyroidism was confirmed in each case, in addition to the clinical features, by the increased serum thyroxine (T_4) and tri-iodothyronine (T_3) levels. The material of subclinical hyperthyroidism comprized patients with autonomous adenomas. In the patients of this group, all of whom were euthyroid, the scintiscan had revealed a "hot" nodule in the thyroid. Serum total T_4, T_3, "free" T_4 and TBG had been found normal. The patients were divided on the basis of preliminary studies [20] into two groups. In Group "A" the serum-"free"-T_4, T_4/TBG, T_3/TBG were normal and serum-TSH increased in response to TRH stimulation (200 μg i.v.); in Group "B" the values of "free"-T_4, T_4/TBG, T_3/TBG showed a slight increase within the normal range and there was no TSH-response to TRH stimulation. The patients belonging to Group B were considered to have subclinical hyperthyroidism.

In all cases of primary hypothyroidism, serum total T_4 was less than 40 nmol/l and serum-TSH in excess of 30 mU/l. The patients with subclinical hypothyroidism were clinically euthyroid, the values of serum-total T_4 and T_3 were normal and the serum TSH value exceeded 5.0 mU/l.

The various stimulation tests were performed at 8 h in the morning after an overnight's fast. 5 euthyroid patients were stimulated with an i.v. dose of 200 μg TRH, and blood samples were taken for TSH measurement at 0, 20, 60 and 120 min. 7 days later the test was repeated by administering 10 mg domperidone i.v. 30 min before TRH-stimulation.

The dose for the domperidone test was 10 mg i.v. (Motilium, Gedeon Richter Ltd., Budapest), for the sulpiride test 100 mg of the drug i.m. (Institute of Pharmaceutical Research, Budapest). Blood samples were taken in all cases at 30 min and immediately before administration of the drugs, the average of the two results being taken as baseline value, and subsequently at 30, 60, 120 and 180 min.

For the l-dopa test the patients received 500 mg l-dopa (Dopaflex, EGYT, Budapest) by mouth. The samplings were done as above. In a number of cases the test was repeated during propranolol (Chimimport, Bucharest) treatment with daily doses of 4 times 40 mg (= 160 mg/day) for 6 days, and the l-dopa test was repeated on the morning of the seventh day.

The following determination methods were used: T_4 (Hungarian Academy of Sciences, Isotope Institute; RK-12; normal range: 70—160 nmol/l); T_3 (Hungarian Academy of Sciences, Isosope Institute; RK-11; normal range: 1.2—3.0 nmol/l; TSH (Byck-Mallinckrodt, RIA-mat-TSH, normal range: <1.25—3.8 mU/l); "free" T_4 (RCC; normal range 9.7—24.0 pmol/l TBG (Hoechst; normal range: 12—28 mg/l); GH (Serono; normal range: 0—5 ng/ml; 1 ng = 2 μU WHO 66/217); prolactin (Serono Prolactin-TER; normal range: 5—25 ng/ml; 1 ng PRL-TER standard = 23 μU WHO 75/504; cortisol (Bio-Rad; Quantimmune Cortisol RIA, normal range: 4.5—18 μg/dl = 0.12—0.50 μmol/l).

In consideration of the episodic character of pituitary GH secretion [15], the increase in GH concentration in response to stimulation test could not be regarded as positive unless Δ GH (difference between maximum serum GH and baseline value) was in excess of 5 ng/ml.

The samples were stored at —20 °C until use, and the serum samples of each patient were assayed simultaneously. The tables represent the mean values with the standard errors (S.E.M.). For statistical analysis, Student's *t*-test was used.

Results

Figure 1 represents the TSH response to TRH (200 μg i.v.) and to domperidone (10 mg i.v.) + TRH (200 μg i.v.) in 5 euthyroid patients. On the evidence of the findings, the response of TSH to TRH was considerably enhanced by previous administration of domperidone.

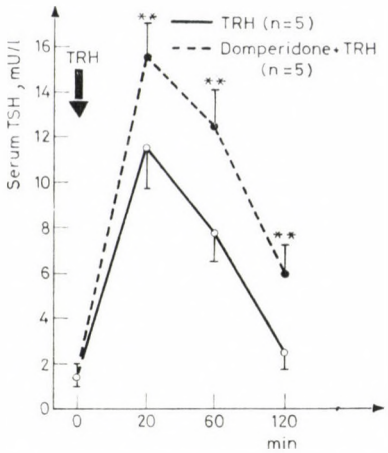

Fig. 1. Response of serum TSH level to TRH and to domperidone (10 mg i.v.) + TRH (200 μg i.v.), in healthy females. ——— TRH; - - - - - - - domperidone + TRH; ✳✳ = $p < 0.01$

As it can be seen, on Table I th response of TSH to domperidone stimulation in subclinical hypothyroidism (7.12 ± 1.48 vs. 1.57 ± 0.151 mU/l Δ TSH; $p < 0.01$), and in manifest primary hypothyroidism (3.48 ± 0.54 vs.1.57 ± 0.151 mU/l ΔTSH; $P < 0.01$) was more marked than in euthyroid patients. Also, after administration of domperidone, serum TSH showed a greater increase in subclin- ical than in manifest primary hypothyroidism (7.12 ± 1.48 vs. 3.48 ± 0.544 mU/1 Δ TSH; $P < 0.05$).

Table II lists the results of thyroid function tests in euthyroid patients with autonomous adenoma. These corresponded in both Group A and Group B to euthyroid values. Although in Group B the values of free-T_4, T_4/TBG and T_3/TBG were within the normal range, they were significantly ($P < 0.05$) above the corresponding values of Group A.

Table I

Response to domperidone (10 mg i.v.) of serum TSH level in female patients with euthyroidism subclinical hypothyroidism and primary manifest hypothyroidism (mean ± S.E.M.)

	Serum TSH mU/l basal value			Δ TSH		
Euthyroidism n = 12	P<0.01	1.53±0.32		P<0.01	1.57±0.15	
Subclinical hypothyroidism n = 9		12.81±3.08	← P<0.001		7.12±1.48	P<0.01
Primary, manifest hypothyroidism n = 14	P<0.001	43.41±3.97		P<0.05	3.48±0.54	

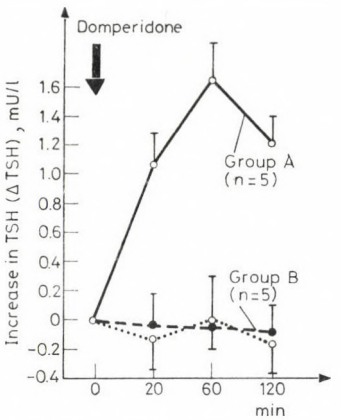

Fig. 2. Response of serum TSH level to domperidone (10 mg i.v.) in two groups of euthyroid patients with autonomous adenoma (see text). ——— Group A; - - - - - - - Group B; ....Control group treated with physiological NaCl

Figure 2 represents the response of TSH to domperidone stimulation in subclinical hyperthyroidism. While in Group A the response of TSH was similar to that found in the controls, in Group B the serum TSH level failed to rise in response to domperidone.

Subsequently the role of dopamine regulation in GH release was examined. Under the effect of l-dopa (500 mg by mouth), a significant increase in

Table II

Results in two groups of patients with euthyroid autonomous adenoma (see text)

	T_4 nmol/l	T_3 nmol/l	Free-T_5 pmol/l	T_4/TBG nmol/mg	T_3/TBG nmol/mg
Group A, n = 5	102.2±6.5	1.65±0.09	16.22±1.05	4.71±0.31	0.86±0.005
Group B, n = 5	128.2±7.8	2.32±0.12	22.30±2.17	5.86±0.480	1.08±0.007
P: A *vs.* B	N.S.	N.S.	<0.05	<0.05	<0.05
Normal limits	70—160	1.2—3.0	9.7—24.0	4.30—5.90	0.080—0.110

Table III

Response of serum GH level to l-dopa (500 mg orally)

	Δ GH ng/ml	
Euthyroidism (n = 5) Phys. NaCl	2.06±0.72	P<0.01
Euthyroidism(n = 6)*** l-dopa	20.80±4.11	
Hyperthyroidism (n = 10)** l-dopa	6.25±1.85	P<0.01
Pr. hypothyroidism (n = 7)**– l-dopa	7.16±2.19	

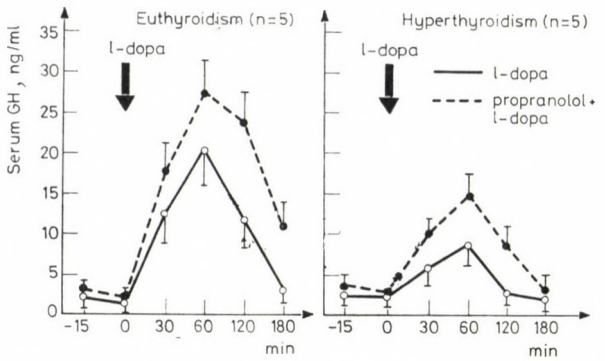

Fig. 3. Response of serum GH level to l-dopa alone (———), l-dopa after previous administration of propranolol (- - - - -) in euthyroid and hyperthyroid female patients

the serum GH level occurred, but Δ GH was significantly lower in the hyperthyroid and the hypothyroid than in the euthyroid group (6.25 ± 1.85 vs. 20.80 ± 4.11, and 7.16 ± 2.19 vs. 20.80 ± 11 ng/ml; $P < 0.01$).

The data presented in Fig. 3 indicate that pretreatment of euthyroid subjects with propranolol (160 mg/day) was followed by an enhanced GH-response to l-dopa. This was also valid for the hyperthyroid group. Yet, at the time of propranolol treatment the maximum GH-response to l-dopa was significantly weaker than in the controls ($P < 0.01$).

In the euthyroid group ($n = 12$) the increase in serum GH concentration in response to sulpiride stimulation was not significant. On individual exami-

Table IV

Response of serum GH and prolactin level to sulpiride (100 mg i.m.) (n = 11)

Sulpiride	Time (min)			
	basal value	30	60	120
GH ng/ml	1.64 ± 0.276	3.22 ± 1.296	2.19 ± 1.305	1.41 ± 0.417
Prolactin ng/ml	8.8 ± 1.43	235.7 ± 24.62	148.1 ± 17.51	79.0 ± 12.01

Table V

Response of serum GH level to domperidone (10 mg i.v.) in euthyroid and hyperthyroid female patients

Domperidone	Serum-GH (ng/ml)	
	Euthyroidism (n = 14)	Hyperthyroidism (n = 10)
	1.84 ± 0.67	2.17 ± 0.51
30 min	1.72 ± 0.75	1.42 ± 0.35
60 min	1.25 ± 0.30	1.32 ± 0.34
120 min	1.50 ± 0.41	2.45 ± 0.52
180 min	1.04 ± 0.39	2.67 ± 0.52

nation of the controls a significant increase in the serum GH level in response to sulpiride was found in 2 of our 12 cases, Δ GH being in excess of 5 ng/ml, whereas in the other cases the change failed to exceed 5 ng/ml. The serum PRL concentration increased significantly after administration of sulpiride in all of the cases.

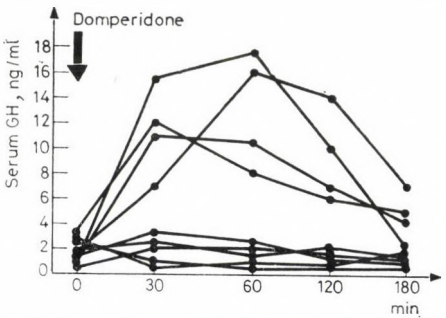

Fig. 4. Response of serum GH level to domperidone (10 mg i.v.) in female patients with primary hypothyroidism

Domperidone (10 mg i.v.) failed to affect the serum GH level to any significant degree either in the euthyroid (n = 14) or in the hyperthyroid (n = 10) group. In the controls the GH level showed a slightly declining tendency, whereas in the hyperthyroid group a transitory decrease was followed by a slight increase. The changes in serum GH were, however, not significant in any of the groups and Δ GH remained below 5 ng/ml.

According to Fig. 4, domperidone caused a significant increase in the serum GH level in 4 out of 9 patients with primary hypothyroidism.

Discussion

It has been confirmed in recent years that the control of TSH-secretion involves, in addition to TRH-stimulation and feedback mechanism, also a dopaminergic regulation [33], manifested with an inhibition of tonic character of TSH secretion, in all probability by enhancing the negative feedback regulation, and affecting the circadian variations of TSH secretion [36]. The findings according to which the release of TSH from the pituitary increases in response to dopamine receptor blockers and that the responses vary in intensity with the functional state of the thyroid [16, 34] are consistent with a dopaminergic inhibitory mechanism. Correspondingly, administration of these drugs is followed by a slight increase in serum TSH even in euthyroid subjects, by a more marked increase in manifest primary hypothyroidism,

and the reaction has been found to attain its maximum in subclinical hypothyroidism. These earlier observations have been supported by the present findings, according to which the increase in the serum TSH level in response to TRH stimulation was more marked in the subclinical than in the manifest, primary form of hypothyroidism.

Since we lack any information on the mechanism by which acute dopamine inhibition operates in subclinical hyperthyroidism, we have extended our studies to clinical cases of this type. This group was formed by euthyroid patients with autonomous thyroid adenoma, in whom serum total T_4 and T_3 were normal, while the values of "free" T_4 and of the T_4/TBG- and T_3/TBG-quotients, though still within the normal range, were slightly increased, and TRH failed to elicit a TSH-response. In all these cases domperidone failed to affect the serum TSH level. This seems to suggest that, similarly to the absence of the TRH → TSH-response, the response of TSH to dopamine-blocking agents also failed to appear even though the serum thyroid hormone levels are but slightly increased without exceeding the normal range. All this demonstrates that the dopaminergic regulation of TSH release is modulated by the intensified negative feedback-mechanism at a high level of sensitivity.

The present results seemed to confirm earlier findings [14, 29] in that the dopaminergic system may influence TSH secretion also by acting directly on the pituitary. This is supported by the observation that domperidone which fails to pass the blood-brain barrier, enhanced TSH release from the pituitary in vivo, and that previous administration of domperidone results in an intensified TSH response to TRH. Recent experimental results in vitro also point to a direct dopaminergic regulation of the pituitary. In agreement with these findings the presence of hypophyseal dopamine receptors has been demonstrated [21], moreover, on the evidence of studies in vitro, dopamine has been found to reverse the stimulating effect of TRH on TSH secretion [3]. These observations are, however, by no means exclusive of the part played by the central, dopaminergic mechanism in the regulation of TSH secretion.

The pituitary release of GH is under the control of two hypothalamic hormones, the stimulating GHRF (growth hormone releasing factor) and the inhibitory somatostatin [23]. For the formation of GH, thyroid hormones are also required [39]. Certain observations suggest, though, that T_4 and T_3 act as suppressors of GH secretion. The dopaminergic neurotransmitter system also takes part in the physiological regulation of GH secretion. Under physiological conditions dopamine (DA) seems to play a complex part in this process: while stimulating basal GH secretion, it depresses the enhanced GH secretion elicited by various stimuli [3, 4, 28, 38]. Changes in thyroid function are accompanied by modifications in the number of pituitary DA-receptors [21]. It is, therefore, uncertain in what manner dopamine regulation affects the

secretion of GH in hyperthyroidism and also in hypothyroidism. We have attempted to answer this question in a further series of studies by stimulation tests with the dopamine agonist l-dopa, and with the dopamine receptor blocker of "peripheral" action, domperidone, with subsequent follow-up of the serum GH level. One of the reasons why studies relating to GH have been deemed of importance, is connected with the generally known fact that both hyperthyroidism and hypothyroidism are associated with disorders of glucose metabolism. Abnormalities of the secretion and metabolism of insulin and glucagon in these conditions have been demonstrated in earlier studies [18, 19] and, as indicated by recent evidence, elevation of the serum GH level even within the physiological range affects the peripheral activity of insulin [7].

L-dopa after oral administration is converted in the CNS and at the periphery to dopamine, subsequently to norepinephrine, and in normal individuals it enhances pituitary GH secretion [5]. According to our studies, the increase in the serum GH level in response to l-dopa is slighter in both hyperthyroid and hypothyroid than in euthyroid female subjects. If, therefore, the function of the thyroid is affected, the GH response to l-dopa is weaker than in normal subjects, in a similar manner as the GH response to hypoglycaemia or to stimulation with arginine [8, 9, 22, 25]. Various explanations accounting for the abnormal GH response may be offered, namely, impaired absorption or metabolism of l-dopa, changes in the central trop-hormone functions, changed responsiveness to dopamine, and possibly an impaired hypophyseal GH production, which might be partly attributed to the increased serum T_4 and T_3 levels.

Propranolol is known to intensify the post-hypoglycaemic GH response [30, 40]. According to the present study, propranolol enhances the GH response to l-dopa in hyperthyroidism in the same way as under normal conditions, probably because the stimulating activity of the central norepinephrine system on GH secretion, taking effect via the alpha receptors, is enhanced [27]. It has been found, on the other hand, that in case of treatment with beta-blockers the maximum serum GH level in response to l-dopa is significantly lower in hyperthyroid than in euthyroid subjects, these findings being consistent with earlier observations in hypoglycaemia + propranolol administration. All this suggests that in this disease it is not possible to stimulate pituitary GH secretion in the same degree as in normal subjects. According to our findings, this is in no way connected with feedback-processes elicited by the increased serum GH level, since the basal serum GH concentration of hyperthyroid patients corresponds to that of healthy subjects. The potential role of somatostatin in the production of the deficient GH response has none the less to be taken into account, since, according to earlier studies [6], the increase in the concentration of thyroid hormones provides in itself a stimulus to the intensification of the hypothalamic secretion of somatostatin.

It is easier to account for the impaired GH response elicited by l-dopa in hypothyroidism, since it has been shown in animal studies that in hypothyroidism the hypophyseal GH concentration is lower than the control value [37].

Subsequently the effect of dopamine receptor blockers on GH secretion was examined. First, the question was studied whether sulpiride, of identical site of action as metoclopramide (MCP), having also a central action, and domperidone of a peripheral site of action, affected hypophyseal GH excretion in a similar manner. Earlier data concerning the question how pituitary GH secretion was affected by MCP are inconsistent [2, 10, 11, 35]. According to our findings, sulpiride left the serum GH level unaffected in 10 out of 12 cases. This was clearly at variance with the results of Chiodera et al. [10], according to which the serum GH level of healthy females rises significantly in response to MCP.

Seeking to explain the confusing results, it was suspected first that the sulpiride used might have been inefficient. This possibility, however, had to be rejected, since the serum prolactin level increased in response to sulpiride in all of the cases. In fact, the results of the tests may be affected by individual features of the patients and by differences in the conditions of the study. Some data indicate for instance that the effect of dopamine receptor blockers on GH secretion is modulated by the oestrogen/androgen ratio, a mechanism which may involve the biphasic effect of the oestrogens on GH release [1]. It has also been shown that motor activity, for instance walking, may increase the serum GH level in females, particularly in the luteal phase. In order to eliminate interfering factors of this kind, only hospitalized, endocrinologically normal, non-users of oral contraceptives were studied. The questions posed by the inconsistency of the results have yet to be clarified by further studies.

Similarly to sulpiride which has also a central action, domperidone left the serum GH level unaffected in all euthyroid and hyperthyroid patients with the exception of a single one, while it caused a significant elevation of the serum GH level in a number of patients with primary hypothyroidism. This response pattern is unrelated to any stress factor, since stimulation with domperidone left the serum cortisol level unaffected.

The abnormal response of GH secretion to stimulation with domperidone in hypothyroidism is reminiscent of the effect of TRH. In opposition to euthyroid subjects, in hypothyroid patients the serum GH level in response by TRH is increased [12, 24]. On the evidence of recent studies, in vitro alterations of pituitary responsiveness may alone account for this response pattern [37]. Our studies with domperidone, are also suggestive of a direct effect on the pituitary. On the other hand, involvement of somatostatin in the production of the GH response has also to be considered, since primary hypothyroidism is associated not only with a low hypothalamic concentration of soma-

tostatin, but also with an impaired responsiveness of somatostatin release [6]. To test these hypotheses further studies will, however, be required.

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THE EFFECTS OF BENZODIAZEPINES AS ANAESTHESIA INDUCING AGENTS ON PLASMA CORTISOL LEVEL IN ELECTIVE HYSTERECTOMY

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The effects of anaesthesis inducing benzodiazepines (diazepam, flunitrazepam, midazolam) on serum cortisol level, blood pressure and pulse rate were examined in patients undergoing elective hysterectomy. Diurnal variation of serum cortisol was established two days before operation.

Either form of benzodiazepine provided good suppression of the cortisol response to stress during anaesthesia and surgery. The endocrine response to surgical stress was more pronounced at end of the operation in all three groups.

Considering the relative changes of cortisol compared to the pre-operative values the smallest differences were seen in the group receiving midazolam. At 6 p.m. on the day of operation the cortisol level decreased in the flunitrazepam and midazolam groups. Especially in the patients who received midazolam at 6 a.m. on the following day the values did not differ from those observed in the control group.

On the basis of these examinations it would appear that to prevent adverse stress-induced effects there is a slight advantage in using midazolam for induction of anaesthesia.

Keywords: cortisol, stress, anaesthesia induction, benzodiazepines, hysterectomy

Introduction

The importance of stress occurring in response to a surgical stimulus, and the role of anaesthetics in suppressing this have received much attention [1, 3, 20, 21].

It is clear that events such as endotracheal intubation, surgical incision, and intra-abdominal pelvic surgery cause a release of catecholamines and cortisol in anaesthetized patients and many of these stimuli can be attenuated by various anaesthetics and autonomic blocking agents [11, 19]. Various approaches to "stress free" anaesthesia are being popularized in an attempt to minimize potentially harmful hormonal and metabolic changes caused by excessive surgical stress [8, 9, 14, 16].

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Benzodiazepines are widely used for inducing anaesthesia. Following studies on the metabolism clinical use of diazepam and Ro-21-3981 extending over many years, the studies have been continued using midazolam [4, 6, 21]. Midazolam is a new benzodiazepine which differs basically from diazepam and flunitrazepam in that it is water soluble and has a shorter half-life [4, 6].

This study examined three different kinds of benzodiazepines concerning their stress attenuation during the same surgical procedure. The response to stress was assessed by monitoring plasma cortisol, blood pressure and heart rate, midazolam was compared to diazepam and flunitrazepam for stress-lowering effect.

Material and methods

Three benzodiazepine derivatives were investigated in 37 ASA class 1 (The classification of physical status adopted by the American Society of Anaesthesiologists, Class 1—5) patients undergoing general anaesthesia. The patients were scheduled for elective hysterectomy by laparotomy due to fibromyomas.

They were divided into three groups which each received a different benzodiazepine as an anaesthesia inducing agent. A few important clinical data aresummarized n T able I.

Table I

Clinical data of patients who underwent hysterectomy

Groups benzodiazepines number of patients	Age (yr) from — to	Weight (kg)	Duration of surgery min.	Duration of anaesthesia min.
Diazepam n = 14	29 —52 45.1 ± 1.79	66.89 ± 2.95	79.57 ± 4.70	99.86 ± 4.07
Flunitrazepam n = 12	33 —54 44.5 ± 1.98	65.75 ± 3.93	81.83 ± 7.08	107.66 ± 8.86
Midazolam n = 11	33 —55 45.54 ± 1.95	63.3 ± 2.39	87.72 ± 7.78	107.27 ± 8.29

mean ± S.E.M.

Premedication, induction of anaesthesia and dosage of different drugs were standardized according to body-weight (Table II). The evening before operation the patients were premedicated with glutathimide (Noxyron^R) and diazepam (Seduxen^R). On the morning of the operation, diazepam (0.16 mg/kg) was administered and 30 min before surgery, pethidine (Dolargan^R) (0.80 mg/kg) and atropine (0.0080 mg/kg), intramuscularly. Anaesthesia was induced between 8 and 10 a.m. and endotracheal intubation was facilitated by succinylcholine 1 mg/kg. Anaesthesia was maintained with a 4/2 nitrous oxid/oxygen mixture. Pancuronium bromide (Pavulon^R 0.06 mg/kg) was given when necessary. Adequate ventilation was maintained by artificial respiration. Arterial blood pressure and heart rate were monitored throughout.

From the group which received diazepam, four blood-samples were taken, two days before operation at 6 a.m., 12 noon, 6 p.m., and the next morning for the purpose of determining diurnal variation of the cortisol level. These values served as controls during the day of operation.

Measurements were carried out on venous samples drawn at 6 a.m.; prior to induction of anaesthesia; following intubation; 5 min after skin incision; at the end of operation; after extubation, i.e. when the patient was able to respond to questions; at noon; 6 p.m.; and 6 a.m.

Table II

Dosages of different benzodiazepines and narcotic-analgetic drugs administered for anaesthesia induction

Groups benzodiazepines number of patients	Diazepam mg/kg	Flunitrazepam mg/kg	Midazolam mg/kg	Fentanyl μg/kg	Thiobutabarbital mg/kg
Diazepam n = 14	0.23 ± 0.03	—	—	3.97 ± 0.67	3.93 ± 0.43
Flunitrazepam n = 12	—	0.015 ± 0.001	—	2.14 ± 0.21	2.84 ± 0.32
Midazolam n = 11	—	—	0.29 ± 0.038	4.42 ± 0.52	—

mean ± S.E.M.

next morning. The samples were centrifuged and the serum was stored at —30C° until analysed. Cortisol was measured by radioimmunoassay [22].

All three groups were comparable concerning the anaesthetic management. Results presented in the text, Tables and Figures are expressed as mean values ± SEM. The data were analysed by different tests; $P < 0.05$ was considered statistically significant. Student's *t* and *d* as well as Wilcoxon's non-parametric test were used for comparison of paired data. Among several groups the examination of expectable cortisol values were carried out using the Kruskal—Wallis test and one-way analysis of variance. The relative changes of cortisol compared to the starting values were examined by quotient-test. Kruskal—Wallis test and variance analysis were also used to analyse the relative changes of cortisol characteristic of the same intra-operative time in the different benzodiazepine groups.

Results

The cortisol level on the day before operation exhibited characteristic diurnal variation within the normal range (Fig. 1). The values at 6 a.m., 12 noon and 6 p.m. in the control group corresponded to those in the literature and (391.2 ± 51.29,226.0 ± 34.78, 146.5 ± 12.63 nmol/l, respectively).

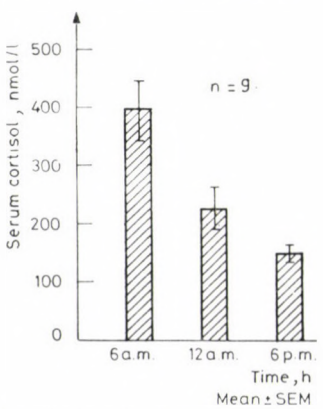

Fig. 1. Diurnal variations in plasma cortisol level in 9 women

In Fig. 2 are shown the corticol concentrations in the three series during the entire anaesthesia and operative periods. Cortisol values measured before induction of anaesthesia were taken as 100% and values during anaesthesia and operation were compared to this.

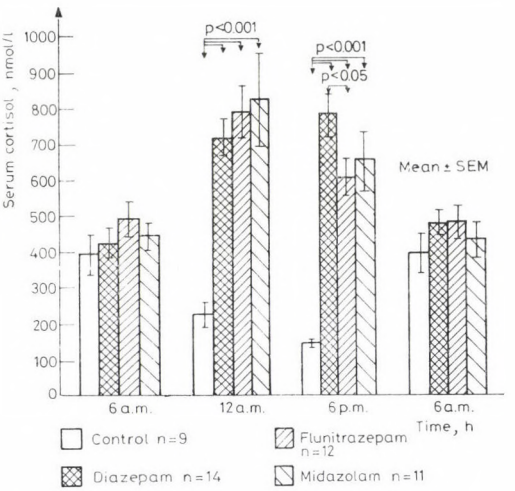

Fig. 2. Cortisol level during anaesthesia and surgery with the administration of different benzodiazepines

The cortisol level increased slightly (106.7 ± 9.4%) following intubation in the diazepam group. Values measured 5 min after skin-incision differed moderately (131.6 ± 14.6%, $P < 0.05$) but at the end of operation (202.2 ± ± 20.8%) and after extubation (199.6 ± 22.7%) considerably ($P < 0.001$), from the pre-induction values (Fig. 2).

In the flunitrazepam group the plasma cortisol decreased after intubation (91.7 ± 6.7%), which continued 5 min after incision (85.5 ± 5.6%) and this difference was slightly significant ($P = 0.05$) compared to the starting value. At the end of operation (218.2 ± 29.7%, $P < 0.01$) and following extubation (230.7 ± 27.7%, $P < 0.001$), high cortisol values were observed (Fig. 2).

In the group which received midazolam (Dormicumr) the cortisol level slightly decreased after intubation (96.7 ± 3.9%). Túe values after incision were similar to that measured before induction of anaesthesia (100.8 ± 10.8%). At the end of operation and following extubation (189.1 ± 23.4%) a significant ($P < 0.01$) increase (207.6 ± 28.9%) was noted (Fig. 2).

Plasma cortical levels at 12 noon were considerably higher ($P < 0.001$) in all three groups (diazepam 717.00 ± 51.47, flunitrazepam 794.08 ± 70.58, midazolam 825.36 ± 128.99 nmol/1) than the control values (Fig. 3).

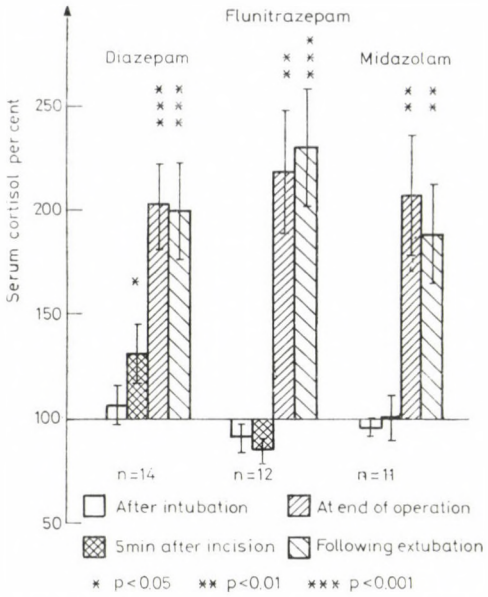

Fig. 3. Cortisol values on the morning of the day of operation, after surgery at noon, 6 p.m., and next morning

At 6 p.m. the cortisol values further increased in the diazepam group (785.78 ± 54.37 nmol/l), but in the other two groups a decrease was observed (flunitrazepam 610.08 ± 51.07, midazolam 656.0 ± 72.75 nmol/l). The difference was significant ($P < 0.05$) when the diazepam and flunitrazepam groups were compared.

Cortisol levels measured at 6 a.m. on the following day (diazepam 483.43 ± ± 37.49, flunitrazepam 481.91 ± 42.39, midazolam 435.36 ± 44.35 nmol/l) almost corresponded to the values at 6 a.m. before operation, and these values were within the normal range (391.2 ± 51.29 nmol/l), (Fig. 3).

Benzodiazepines were administered according to body-weight. Fentanyl was fractionally given until narrowing of the pupil had set in. It was found that the required dose of fentanyl for causing this sign was different in the three groups. The smallest dose was necessary in the patients receiving flunitrazepam. For disappearance of the eyelash reflex no thiobutabarbital was required in the midazolam group.

Blood pressure and pulse rate were compared to values measured before induction ofanaestúesia. Considering that a change of 10 mmHg (respectively 10(min) cannot be considered a difference, deviation from 10 of the absolute values of changes were presented (Δ blood pressure, Δ pulse rate), (Fig. 4).

The most significant decrease in blood pressure approximately 20 mmHg was observed after intubation. At the end of the operation blood pressure was

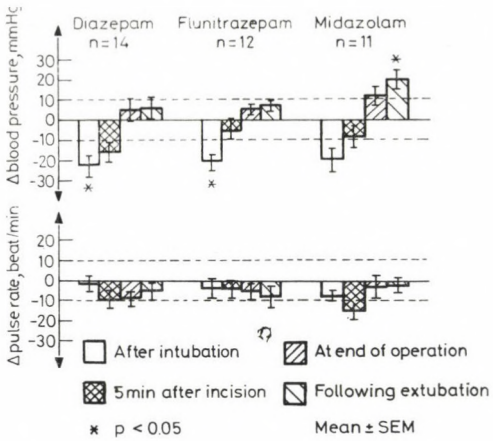

Fig. 4. Blood pressure and pulse rate during anaesthesia and surgery with the administration of different benzodiazepines

increasing but its increase did not exceed 20 mmHg, even in the midazolam group.

Pulse rate showed no significant decrease in any of the three groups.

In the postoperative period, blood pressure and heart rate were stable in all three groups.

Discussion

Surgical procedures evoke an endocrine response which results in substrate mobilization, a change in metabolism towards catabolism with a negative nitrogen balance and retention of salt and water. The magnitude of this response is proportional to the severity of the operative trauma [3, 17]. Major surgical procedures in human beings result in an increase in plasma cortisol [2, 5, 8, 12]. The only known physiologic stimulus for adrenal secretion of cortisol is ACTH and htis trophic hormone has been found to be elevated by one-balf hour after beginning of surgery [5].

Túe most important emotions in the pre-operative period are anxiety, depression and astgenia. They are part of human stress reactions which also involve hormonal (ACTH, cortisol, etc.) and sympathetic activity. A rise in plasma cortisol is seen in stress situations such as pelvic gynaecological or indeed any major surgery, thus documenting that it is an important stress parameter.

There are few data available about how benzodiazepines influence surgical stress and within this adrenal cortisol production during major surgery. To evaluate stress attenuation during anaesthesia, measurement of stress hormones

(cortisol, catecholamines) has been established to be reliable parameters [1, 3, 11, 15, 22]. The sympathetic nervous system is activated by a variety of events which occur during anaesthesia and surgery. Most plasma noradrenaline comes from sympathetic nerve terminals and most adrenaline from the adrenal medulla. These and the cortisol produced by the adrenal cortex are principal humoral markers of the multiple stresses of anaesthesia and surgery.

Anaesthesia with fentanyl, oxygen and nitrous oxide is currently a popular technique and an alternative to halogenated agents. Stanley at al. [18] have proposed the use of large fentanyl doses to prevent liberation of catecholamines and cortisol. The present paper reported on the cortisol response major gynecological surgery during anaesthesia induced witú benzodiazepines administered iv. in clinically used doses.

Benzodiazepine derivatives are widely used for a variety of clinical indications [7, 10]. The characteristic effects of the benzodiazepines are their anticonvulsant, anxiolytic, sleep-inducing, muscle relaxant, and sedative actions. Midazolam is a new benzodiazepine whicú, when compared with its predecessors diazepam and flunitrazepam has several advantages [4, 6, 13]. The imidazole ring confers to the molecule a higher basicity, a high stability in solution and a short duration of action. Midazolam is water-soluble and has been studied as an anaesthesia induction agent [4, 6]. It could also be used as a component of balanced anaestúesia to provide sedation and amnesia as well as to prevent the stressinduced adverse hormonal and metabolic changes. Since diazepam and flunitrazepam have been used for all these purposes, comparative observations were conducted on the effects of these benzodiazepines on the plasma cortisol level.

Clinical and endocrine investigations have demonstrated the expected effectiveness and advantages of benzodiazepines in anaesthesiology [4, 13]. Measurement of the cortisol level could provide further details when new anaesthesia inducing agents are introduced into practice.

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Cardiology

POST-EXERTION CHANGES IN LEFT VENTRICULAR SYSTOLIC TIME INTERVALS IN PATIENTS WITH PRIMARY HYPERTENSION TREATED WITH HYDROCHLOROTHIAZIDE, BINAZINE, AND PROPRANOLOL

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The study involved 13 patients with primary hypertension who exercised on a bicycle ergometer with intensity increasing up to submaximum level. The exercise was carried out in four stages: before treatment (1st study), following one week treatment with 50 mg hydrochlorothiazide daily (2nd study), after one week treatment with the same dose of hydrochlorothiazide and 120 mg binazine daily (3rd study), and after one week treatment with hydrochlorothiazide and binazine, and 60 mg of propranolol daily (4th study). Using the approach of Weissler et al., left ventricular systolic time intervals were analysed at rest, after exercise and up to the 90th minute of restitution. Hydrochlorothiazide and binazine treatment decreased systolic and diastolic blood pressure, the total electromechanical systolic time index (QS_2I) and the left ventricular ejection time index (LVETI), and increased the PEP/LVET index at rest and after exercise. Addition of propranolol did not augment the hypotensive effect, while the left ventricular systolic time intervals returned to the values observed before treatment.

Keywords: hypertension, physical effort, left ventricular dynamics.

Abbreviations: Ps: systolic blood pressure, Pd: diastolic blood pressure, QS_2I: electromechanical systolic time index, LVTI: left ventricular ejection time index, PEP: preejection period.

Introduction

Haemodynamic disturbance accompanying systemic hypertension continue to attract much interest. It has been found that in the early phase of hypertension an increase in cardiac output and stroke volume takes place with the total vascular resistance remaining unchanged [4, 6, 12, 24]. It is believed that cardiac contractility during that time increases or remains unchanged,

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while left ventricular end-diastolic volume and pressure increase [6, 18]. Stable systemic hypertension involves increased peripheral resistance, decreased cardiac output, gradual hypertrophy of the heart muscle, and decreased left ventricular diastolic volume and pressure [6, 12, 23, 24]. The literature contains conflicting reports on the inotropic state of the hypertrophic heart muscle [19, 25]. Disturbed contractility has been observed primarily in patients with stable systemic hypertension after they had performed some physical effort [1, 29]. Over the last several years ergometric tests have been applied for diagnosing the early phases of hypertension [15, 22], to assess physical efficiency [2, 17], and to study the effectiveness of hypotension treatment which, it is thought, should prevent an excessive rise of pressure during physical exertion [9, 10]. The aim of the present study was to investigate the changes in post-exertion left ventricular dynamics in the course of hypotension treatment in patients suffering from primary hypertension.

Material and methods

The study involved 13 patients with primary hypertension. The diagnosis and the investigations were carried out under clinical conditions. The group consisted of 7 women and 6 men aged 42 to 68, mean age 51.5 ± 7.8 years, all of whom were found to be in the second period (according to the WHO classification) of stable systemic hypertension. On admission the patients had a mean systolic blood pressure of 173.1 ± 15.1 mm Hg and a diastolic pressure

Table I

General characteristics of hypertensive patients who exercised on bicycle ergometer before treatment (3), and hydrochlorothiazide,

No.	Name	Sex	Age	Height, cm	Body weight, kg	Minnesota code
1.	D. Cz.	M	57	165	87.7	II-1
2.	G. E.	M	68	168	72.0	II-1; IV-1; IX-3
3.	K. M.	M	57	173	72.5	II-1; V-3
4.	P. F.	M	50	173	98.0	VII-4; IX-1
5.	Z. M.	M	47	172	76.0	II-1; III-1
6.	H. K.	F	53	157	70.8	II-1; IV-3
7.	N. T.	M	52	167	72.6	III-1; IX-3
8.	G. T.	F	49	146	62.0	V-3
9.	J. T.	F	48	158	89.1	0
10.	O. B.	F	45	160	67.0	II-1; IV-3; V-3
11.	Ch. J.	F	42	167	85.4	IV-2
12.	Z. W.	F	44	158	77.7	II-1; IV-3; V-2; IX-3
13.	S. W.	F	58	162	74.0	II-1; V-3
	mean		51.5	163.5	77.3	
	±SD		7.8	8.1	10.8	

of 103.1±12.0 mm Hg. They received no drugs for at least seven days prior to the study. Following that they exercised on a Zimmermann bicycle ergometer (GDR) at 60 revolutions per minute (1st study). Initial intensity of the exercise was 30 Watt (W) and this was increased by 30 W every five minutes. The successive phases of the exercise were separated by 3-minute intervals during which polycardiograms were made. The intensity of the effort was from 30 to 120 (mean 78.5±27.1) W and the work performed ranged from 8976.1 to 89761.4 Joule (mean 46952.1±24316.3 Joule). Heart rate (HR) acceleration was from 116 to 180/minute, mean 146.2±19.3/minute. Exercise was discontinued when submaximal HR was attained. In two cases the exercise was discontinued earlier when symptoms of myocardial ischaemia were noted in the electrocardiogram.

Following the first study, hypotension treatment was applied according to the following schedule: hydrochlorothiazide 50 mg daily (2nd study), hydrochlorothiazide 50 mg and binazine (Polfa, Poland) 120 mg daily (3rd study), and hydrochorothiazide 50 mg, binazine 120 mg, and propranolol 60 mg daily (4th study). Each phase of the treatment lasted seven days. Owing to earlier normalization of arterial blood pressure, 12 patients were qualified for the third study, and 11 for the fourth. Following each phase of treatment the subjects performed physical exercise the intensity of which was identical to that performed before treatment. Heart rate acceleration in the 2nd study was from 120 to 188/min, mean 150.5± 20.5; in the 3rd study, 140 to 174/min, mean 149.0±10.2; and in the 4th, from 96 to 156/min, mean 133.1±16.3 (Table I).

Using a three-channel ECG apparatus (Multicard — 3 M) and with the patients lying, synchronous records of the 2nd ECG lead, phonocardiogram from the cardiac apex in the 70 Hz band, and of carotid pulse curve were made before exercise (A), after each phase of the exercise (B, C, D), and in the 5th (E), 10th (F), 20th (G), 30th (H), 60 th (I), and 90th (J) minute of recovery. From the polycardiograms obtained, left ventricular systolic time intervals were calculated using the method of Weissler et al. [28]. In order to exclude the effects of heart rate on total electromechanical systolic time (QS_2) and left ventricular ejection time (LVET) we corrected them according to our own linear regression equations, calculated for each phase of the study, and then this time intervals were presented as indices (QS_2I, LVETI) independent of HR.

The numerical results obtained were subjected to statistical analysis using Student's paired *t* test and considering differences of $P < 0.05$ to be statistically significant [20].

(study 1), after one week of treatment with hydrochlorothiazide (2), hydrochlorothiazide and binazine binazine, and propranolol (4)

Ps/Pd, mm Hg	Maximum load, Watt	Work performed, Joule	Heart rate acceleration			
			1	2	3	4
190/120	90	53 856.8	148	152	156	144
150/80	90	53 856.8	116	120	—	—
155/100	90	53 856.8	116	148	148	—
180/100	120	89 761.4	148	160	140	156
160/100	90	53 856.8	152	144	140	132
170/90	30	8 976.2	128	136	140	96
180/100	120	89 761.4	152	156	156	120
190/100	30	8 976.2	164	168	140	140
170/110	90	53 856.8	140	144	152	130
175/120	90	53 856.8	180	188	174	150
200/120	90	53 856.8	132	136	148	128
170/105	60	26 928.4	164	156	144	140
160/95	30	8 976.2	160	148	150	128
173.1/103.1	78.5	46 952.1	146.2	150.5	149.0	133.1
15.1/12.0	27.1	24 316.3	19.3	20.5	10.2	16.3

Results

Systolic blood pressure (Ps) at rest before treatment was 173.1±15.1 mm Hg, in the second study — 148.5±18.3 mm Hg ($P < 0.001$), in the third study — 142.9±20.5 mm Hg ($P < 0.001$), and in the fourth study it was 145.5±16.3 mm Hg ($P < 0.001$). Exercise produced significant increase of Ps values in the successive studies by 45.0±20.5, 35.1±23.9, 35.4±22.5, and 35.5±18.0 mm Hg ($P < 0.001$) respectively. The extent of Ps increase was in each case similar and non-significant ($P > 0.05$). After exercise, Ps tended to decrease and the decrease continued throughout the entire recovery period ($P < 0.05$). Diastolic blood pressure (Pd) at rest in the first study was 103.1± 12.0 mm Hg, and it decreased by 8.5±13.7 in the second study ($P > 0.05$), by 11.9±12.1 in the third study ($P < 0.05$), and by 9.8±9.3 in the fourth study ($P < 0.05$). Pd values increased non-significantly in all studies during exercise ($P > 0.05$), and decreased during restitution. This decrease was most pronounced at 30 and 60 minute after the exercise and was statistically significant in the 2nd and 4th studies ($P < 0.05$) (Fig. 1).

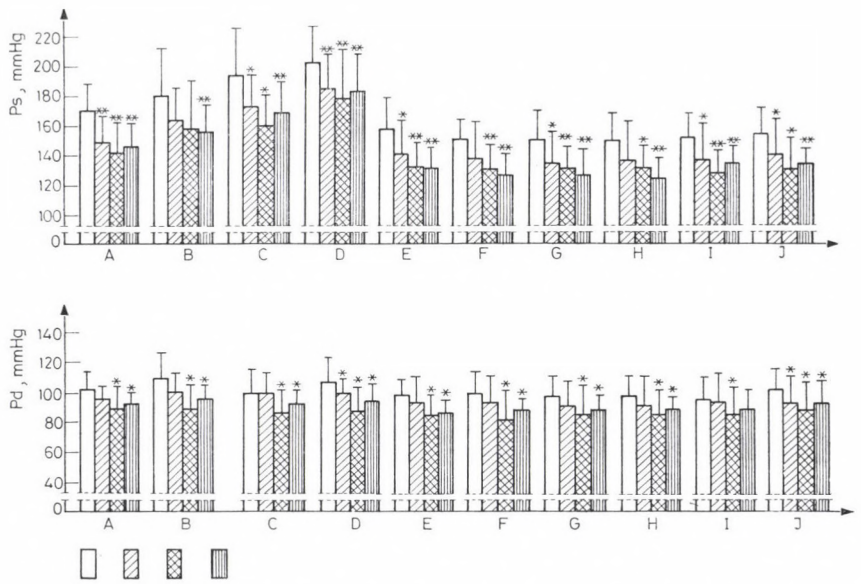

Fig. 1. Systolic (Ps) and diastolic (Pd) blood pressure at rest (A), following exercise with a load of 30 W (B), and 60 W (C) after submaximal effort (D), and in the 5th (E), 10th (F), 20th (G), 30th (H), 60th (I), and 90th (J) minute of restitution. 1 — □ — control study; 2 — ▨ — after one week of treatment with 50 mg/day of hydrochlorothiazide; 3 — ▩ — after one week of treatment with 50 mg/day of hydrochlorothiazide and 120 mg/day of binazine; 4 — ▥ — after one week of treatment with 50 mg/day hydrochlorothiazide, 120 mg/day of binazine, and 60 mg/day of propranolol. * = $P < 0.05$ and ** = $P < 0.001$ in intergroup comparisons

Total electromechanical systolic time index (QS_2I) at rest in the 1st study was 575.7 ± 23.3 ms, and it decreased to 534.3 ± 21.0 in the 2nd study, to 504.1 ± 23.8 in the 3rd study ($P < 0.001$), and in the 4th study it was 574.5 ± 19.0 ms ($P > 0.05$). The most pronounced decrease of QS_2I occurred after submaximal exercise by 24.0 ± 18.7, 20.9 ± 24.1, 34.4 ± 16.9 and 18.5 ± 15.3 ms respectively in the four studies ($P < 0.05$). The degree of QS_2I decrease was similar in all studies ($P > 0.05$). In the course of restitution the lowest QS_2I values were observed in the 2nd and 3rd studies ($P < 0.001$) (Fig. 2).

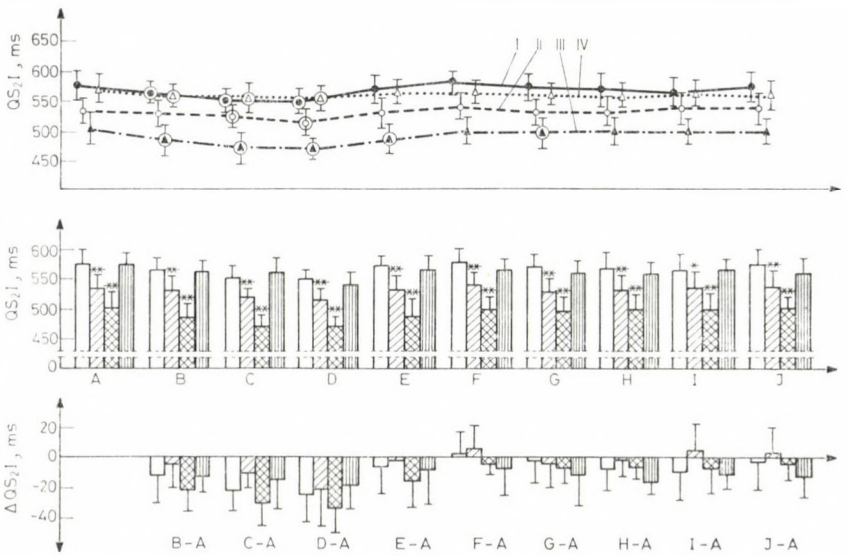

Fig. 2. Total electromechanical systolic time index (QS_2I) in 13 primary hypertensive patient at rest (A), after exertion with loads of 30 W (B) and 60 W (C), after submaximal effort (D) and in the 5th (E), 10th (F), 20th (G), 30th (H), 60th (I), and 90th (J) minute of restitution in the control study (1 — □), after one week treatment with 50 mg/day of hydrochlorothiazide (2 — ▨), after one week treatment with 50 mg/day of hydrochlorothiazide and 120 mg/day of binazine (3 — ▩), and after one week treatment with 50 mg/day of hydrochlorothiazide, 120 mg/day of binazine, and 60 mg/day of propranolol (4 — ▥). From top to bottom: QS_2I values obtained at rest and during exercise and restitution in each study, values obtained in the successive stages of the studies; differences between the results obtained during exercise and restitution, and the initial ones. Statistical comparisons: ○ — $P < 0.05$ (for the dynamics of changes within the studies), and * $P < 0.05$ and ** $P < 0.001$ (for comparison of the results between the studies)

Left ventricular ejection time index (LVETI) at rest in the first study was 422.9 ± 23.4 ms, and it decreased to 391.2 ± 15.2 in the 2nd study and to 383.7 ± 21.9 in the 3rd ($P < 0.001$). In the 4th study the LVETI values was 425.3 ± 18.2 ms ($P > 0.05$). After exercise with an intensity of 60 W and after submaximum exertion the LVETI values in the successive studies were higher,

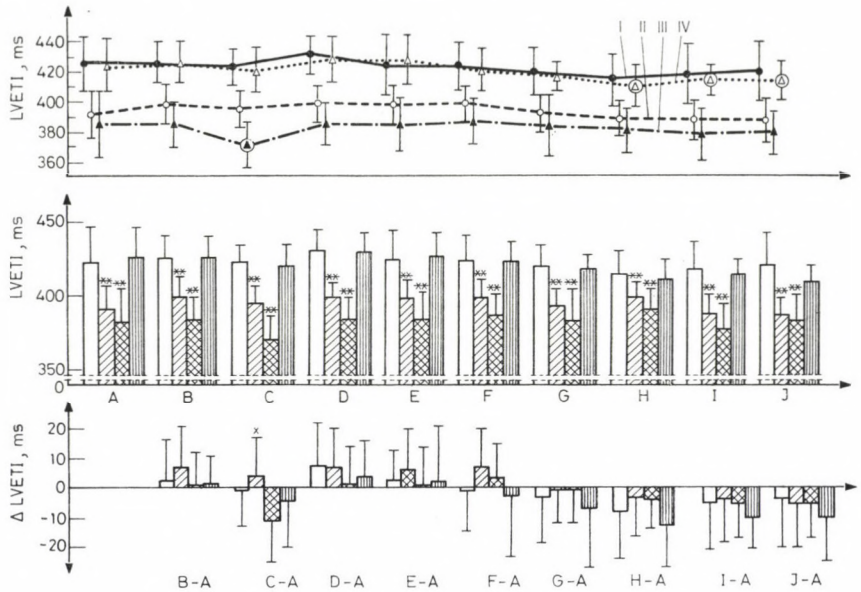

Fig. 3. Left ventricular ejection time index (LVETI) in 13 hypertensive patients during exercise and recovery, before and after treatment. Symbols as in *Fig. 2*

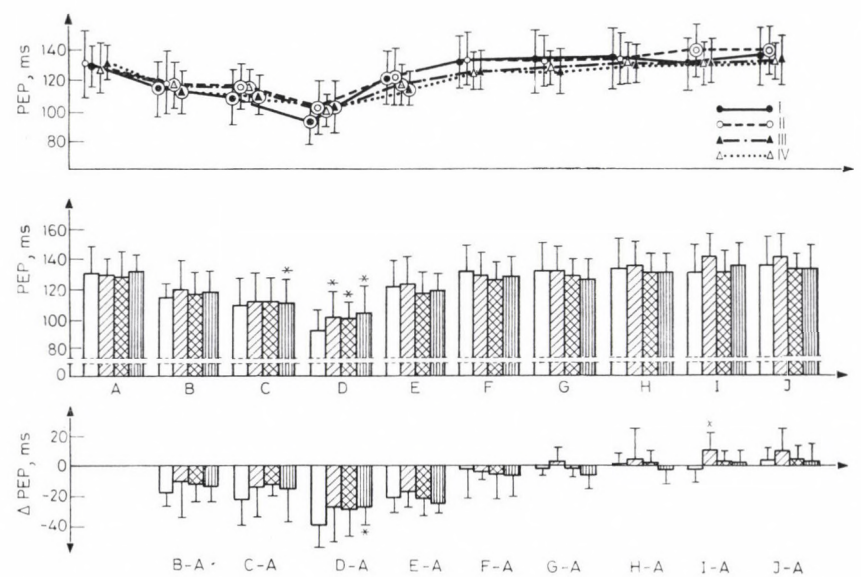

Fig. 4. Preejection period (PEP) at rest, during exercise, and during recovery in hypertensive patients, before and in the course of hypotension treatment. Symbols as in *Fig. 2*

and in the 20th, 30th, 60th, and 90th minutes of restitution lower, than the value obtained at rest ($P < 0.05$) (Fig. 3).

The preejection period (PEP) at rest was in the 1st study 131.6 ± 17.0 ms; in the 2nd study, 129.2 ± 13.4 ms ($P > 0.05$); in the 3rd study, 128.1 ± 16.4 ms ($P > 0.05$); and in the 4th study, 131.0 ± 10.1 ms ($P > 0.05$). In all studies after exercise the PEP values significantly decreased by 41.2 ± 15.3, 27.8 ± 23.6, 28.8 ± 20.6, and 28.2 ± 12.0 ms, respectively ($P < 0.05$). The degree of changes in PEP values in the 2nd, 3rd, and 4th studies was less pronounced than in the 1st study ($P < 0.05$). In the recovery period the PEP values were similar in all studies ($P > 0.05$) (Fig. 4).

The PEP/LVET ratio at rest was in the 1st study 0.485 ± 0.086; in the 2nd, 0.566 ± 0.091 ($P < 0.05$); in the 3rd, 0.527 ± 0.073 ($P > 0.05$), and in the 4th study, 0.486 ± 0.085 ($P > 0.05$). Exercise decreased the PEP/LVET values in the successive studies, but only in the 1st study was the 0.061 ± 0.061 decrease significant statistically ($P < 0.001$). Immediately after the exercise and in the restitution period the PEP/LVET values in the 2nd and 3rd studies were statistically significantly lower than in the 1st study ($P < 0.05$). The dynamics of PEP/LVET changes was similar in all studies ($P > 0.05$) (Fig. 5).

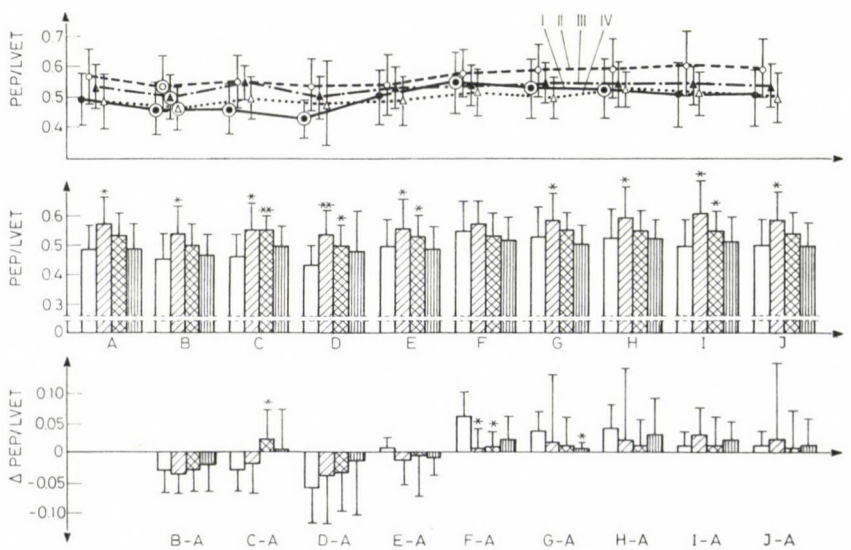

Fig. 5. PEP/LVET ratio in hypertensive patients at rest, during exercise, and during recovery, before and in the course of hypotension treatment. Symbols as in *Fig. 2*

Discussion

Treatment with hydrochlorothiazide applied in a dose of 50 mg/day for 7 days produced a significant decrease in systolic blood pressure and somewhat less pronounced decrease of diastolic pressure. At the same time we observed changes in the left ventricular systolic time intervals: the total electromechanical systolic time index and the ejection period index decreased, while the PEP/LVET ratio increased. The unchanged behaviour of the preejection period and the significantly decreased ejection period index suggested that the basic effect of diuretic treatment was a decreased flow of blood to the heart [3, 13].

Application together with the diuretic of binazine in the commonly used dose of 120 mg/day, caused a dilatation of peripheral blood vessels and brought about a further slight decrease of systolic and diastolic arterial blood pressures and of the QS_2I and LVETI values. There were no significant changes in the preejection period, and the PEP/LVET ratio continued to be non-significantly larger than the initial value. Such a behaviour of the systolic time intervals indicated that left ventricular dynamics continued to be disturbed in the case of combined hydrochlorothiazine-binazine treatment. It was found that drugs which cause dilatation of arterioles give rise to heart rate, increased plasma renin activity, and increased sympathetic nervous system activity and when used alone, an increase in cardiac output [5, 8, 11, 14]. For this reason it is recommended that in such cases drugs blocking the beta adrenergic receptors should be administered [7, 26, 27]. In the present study, administration of 60 mg of propranolol daily, in addition to the previously administered hydrochlorothiazide and binazine, arrested the decrease of blood pressure, and left ventricular systolic time intervals returned to the levels observed before the study.

The research of Martynov et al. [17], and Chalev et Kairov [2], shows that the physical efficiency of hypertensive patients substantially decreases in the early stages of the condition. Following exercise, the patients with primary hypertension showed an increased systolic pressure, which decreased during the recovery period. Ps values were lower than at rest even in the 90th minute of restitution. This behaviour of the systolic blood pressure was similar as that observed in healthy subjects following exercise [16]. Exercise decreased the total electromechanical systolic time index and the preejection period, increased the ejection period index, and decreased the PEP/LVET ratio. We may thus conclude that such a behaviour of the left ventricular systolic time intervals constitutes evidence of proper adaptation of the heart to effort [16]. Between the 10th and 30th minute of restitution a slight deterioration of the left ventricular systolic dynamics occurred.

The direction of changes in systolic and diastolic blood pressure and in left ventricular systolic dynamics during exercise and restitution in the course of hypotension treatment was similar as before treatment. Exercise decreased the QS_2I, PEP, and PEP/LVET values, and increased the LVETI values. It follows that in the course of hypotension treatment the adaptation to effort remained normal [16]. The intensity of the applied exercise did not produce any impairment of heart function during hypotension treatment that included administration of drugs blocking the beta adrenergic receptors. Similarly as during rest, treatment with diuretic and arteriole-dilating drugs during exercise produced a substantial decrease of the ejection time index. Such a behaviour of the ejection index was advantageous because it prevented the excessive accretion of blood pressure during exercise. Addition of a small dose of propranolol caused the systolic dynamics to return to the pretreatment level, with the blood pressure stabilizing at a lower level.

During hypotension treatment, the degree of post-exertion myocardial hypodynamia observed before treatment was distinctly lowered. This may have been related to the lower pressure values and the decreased afterload due to treatment.

Conclusions

1. Combined treatment with hydrochlorothiazide, binazine and propranolol prevented excessive post-exertion increment of systolic and diastolic blood pressure.

2. In comparison with the initial study, treatment with hydrochlorothiazide, hydrochlorothiazide and binazine decreased the total electromechanical systolic time index and the left ventricular ejection time index and increased the PEP/LVET ratio at rest and following exercise: on the addition of propranolol, the left ventricular systolic time intervals returned to the values observed before treatment.

3. In patients with primary hypertension, a disturbance of left ventricular systolic function occurred during recovery. Hypotensive treatment prevented the myocardial hypodynamia syndrome after physical exercise.

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Gastroenterology

THERAPEUTICAL EXPERIMENTS IN ULCERATIVE COLITIS AND CROHN'S DISEASE

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In the period 1963—82 among the patients with aspecific inflammatory bowel disease, the incidence of ulcerative colitis was 3.1 per 100 000 per year, while the frequency of Crohn's disease has doubled during the observation period and now its incidence is 0.58 per 100 000 per year. During the past 20 years, 404 patients with ulcerative colitis were treated. The average follow-up of the patients lasted for 6.6 years. During this period, 40% of the patients could be kept in balance permanently with salicylazosulfapyridine (SASP) monotherapy. A further 34% reacted to SASP plus steroid. The rate of regression was increased by a further 14% when the combination was occasionally completed with a short-term antibiotic or prolonged azathioprine therapy. The inestimable cases and those refractory to treatment made up the other 12% and among them are also the 23 colectomized patients. During the two decades 40 patients with Crohn type ileocolitis were treated. SASP administration by itself was sufficient in only one case among them. In 6 cases steroid, in 4 antibiotics, in 7 azathioprine and in 2 cases metronidazole treatment had to be introduced complementarily. The fact that 21 of the 40 patients had to be subjected to bowel resection in some phase of the disease, shows how impossible it is to evaluate the different therapeutic interventions.

Keywords: ulcerative colitis, Crohn's disease, salicylazosulfapyridine, steroids, azathioprine.

Abbreviation: SASP = salicylazosulfapyridine.

Introduction

The occurrence of inflammatory bowel disease, especially of Crohn's disease, is increasing throughout the world. Since despite widespread research and therapeutical trials the pathogenesis and so the causal treatment of these conditions remains unknown, inflammatory bowel disease is one of the greatest problems of modern gastroenterology. In the following we shall sum up our 20 years clinical experience.

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Patients and methods

In our Gastroenterological Department and Endoscopy Clinic, systemic care of colitis patients has been performed since 1963.

The diagnosis of ulcerative colitis was based in all cases besides the clinical symptoms on rectoscopic and biopsy findings. Earlier, the extension of the process was usually evaluated from radiological descriptions, while since 1977 most of the diagnostic information has been obtained from colonoscopy.

Evaluation of the clinical types of ulcerative colitis was done according to Roth [30]. The pathological activity of the disease was evaluated according to the histological division of Matts [18]. In judging the clinical activity and the grade of severity, we used four categories (Table I).

Table I

Clinical severity and activity grade of ulcerative colitis

Fulminant	Sepsis with > 39 °C fever, profuse purulent-sanguineous diarrhoea, anaemia—hypoproteinaemia
Severe	Toxic symptoms, fever, mucosanguineous diarrhoea > 10 per day, anaemia
Moderate	Moderate toxic symptoms, subfebrility, mucosanguineous faeces < 10 per day
Mild	Mild general symptoms, subfebrility, mild mucosanguineous faeces < 5 per day, sometimes obstipation

For therapy salicylazosulfapyridine (SASP) (Salazopyrin, Pharmacia, Uppsala) was administered as the basic drug initially in large doses (4—6 g per day) then 2 g per day as a preserving dose. Since 1978 we have been using mostly Salazopyridazine (Medexport, Moscow) in a 3 g per day therapeutical or 1.5 g per day preserving dose. In refractory cases or with patients who originally had had severe symptoms, the basic medication was combined by local steroid treatment for a few weeks and in case of extensive disorders by systemic steroid therapy for months. The initial large doses e.g. 40 mg of oral or 100 mg of parenteral

Table II

Activity index of Crohn's disease
(Best et al. [4])

1. Liquid or very soft stools–number in 1 week	x 2 =
2. Quantity of abdominal pains weekly (1 = mild, 2 = moderate, 3 = severe)	x 5 =
3. General well-being weekly (0 = good, 2 = poor, 4 = terrible)	x 7 =
4. Extraintestinal symptoms (fever, joint, eye, mucocutaneous, anal) each of these	x 20 =
5. Diarrhoea abated only with opiates	x 30 =
6. Abdominal mass (0 = absent, 2 = questionable, 5 = yes)	x 10 =
7. Haematocrit: males < 47, females < 42	x 6 =
8. Body weight per cent below standard weight	x 1 =
	Total =

< 150 = rstinge stage, 150—350 moderately active stage, > 350 = seriously active stage

prednisone — depending on the symptoms — were gradually decreased then discontinued. In the lack of a remission, when septic symptoms were dominant, 7 to 10 day antibiotic therapy with gentamycin or clindamycin was introduced, while in case the process was torpid or persistent azathioprine was administered in 100—150 mg daily doses. In refractory cases colectomy was indicated, depending on the clinical state.

According to our therapeutical practice, the patients were kept in hospital during exacerbations. In cases with moderate symptoms or in remission, treatments were performed at the outpatient clinic. Usually clinical control was done in every three months. In case of long-lasting illnesses even if the patient was asymptomatic we have insisted on an annual endoscopic control.

The diagnosis of Crohn's disease was based earlier besides the clinical symptoms on radiological findings, surgical or histological results. Recently, diagnosis is established by colonoscopy and biopsy.

The clinical activity of the disease is evaluated according to the index of Best et al. [4] (Table II). In conservative treatment of Crohn's disease we usually followed the therapeutic scheme used in ulcerative colitis. In refractory cases we occasionally administered other drugs e.g. metronidazole, disodium cromoglycate, penicillamine and levamisole. In both ulcerative colitis and Crohn's disease, depending on the clinical state, drug therapy was completed with dietary and symptomatic treatment. Since for aspecific colitis SASP was used constantly, its side effects were registered continuously.

With the help of other component departments the patients suffering from aspecific inflammatory bowel disease were all collected in Borsod-Abaúj-Zemplén County.

Results

During the 20-year period 1963—1982 we collected 557 patients with aspecific ileocolitis from different hospitals of Borsod-Abaúj-Zemplén County. During the first decade 240 cases of ulcerative colitis were detected and the number of new cases was 3.1 per 100 000 yearly. In the second ten-year period the frequency was the same. As to Crohn's disease, its incidence grew twofold in the second ten-year period, so that the incidence rate of the two inflammatory bowel diseases changed from 10 : 1 to 5 : 1 (Table III).

Table III

Ulcerative colitis and Crohn's disease patients in Borsod-A-Z county during 20 years

1963—1972		1973—1982	
772 000	inhabitants	800 000	inhabitants
Ulcerative colitis incidence:	240 cases 3.1/100 000	Ulcerative colitis incidence:	248 cases 3.1/100 000
Crohn's disease incidence:	23 cases 0.29/100 000	Crohn's disease incidence:	46 cases 0.58/100 000
Rate ~ 10 : 1		Rate ~ 5 : 1	

In the past 20 years we treated 404 patients with ulcerative colitis. Their mean age at manifestation of the diseases was 37.2 years. In the second decade of our survey the initial attack occurred 5 years earlier in average and there was a slight female preponderance. The average follow-up time was 6.6 years.

According to the site of ulcerative colitis, in 52% of the patients it was in the rectosigmoid in 30.5% the process extended to the left part of the colon and in 15% to the whole large bowel. There may have been some unidentified Crohn colitis patients hidden among the cases of segmental inflammation which occurred in 2.5%.

As to its clinical course in 66.5% of the patients the disease was of relapsing, in 20% of chronic continuous and in 6.5% of acute fulminant type. 7% of the patients did not fit in any of the traditional forms, therefore there may have been some unidentifiable Crohn's disease cases in this unclassified group.

As to the grade of clinical severity 52.2% of the patients could be classified as moderate, 22.3% as severe, 19% as mild and 6.5% as fulminant.

As to therapy, 40% of the patients could be treated with SASP alone. 74% of all reacted to SASP plus steroid treatment. In 88% was clinical improvement achieved with these drugs complemented occasionally with antibiotics gentamycin, clindamycin or in refractory patients with chronic azathioprine administration. Refractory cases made up the other 12%. The 23 operated patients belonged to the latter group (Fig. 1).

Significant differences could be seen when analysing the patients according to the therapeutical periods (Table IV). Until 1971 we mostly used SASP alone and occasional enemas. Perforation and other complications made us abstain from systemic steroids. In that therapeutical period the number of refractory cases reached 15%. From among the latter cases 8 patients had to be operated on, but considering the number of those who died a considerably greater number ought to have been operated.

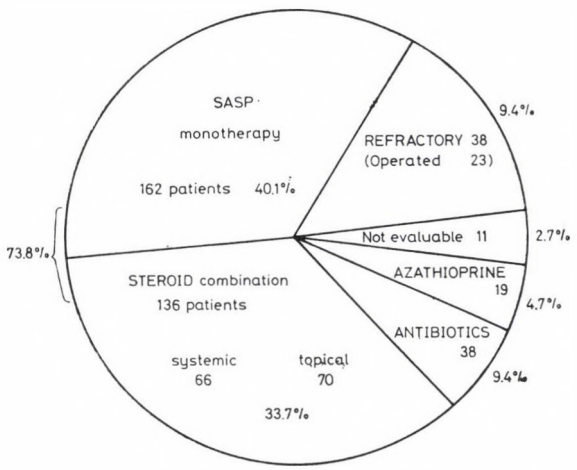
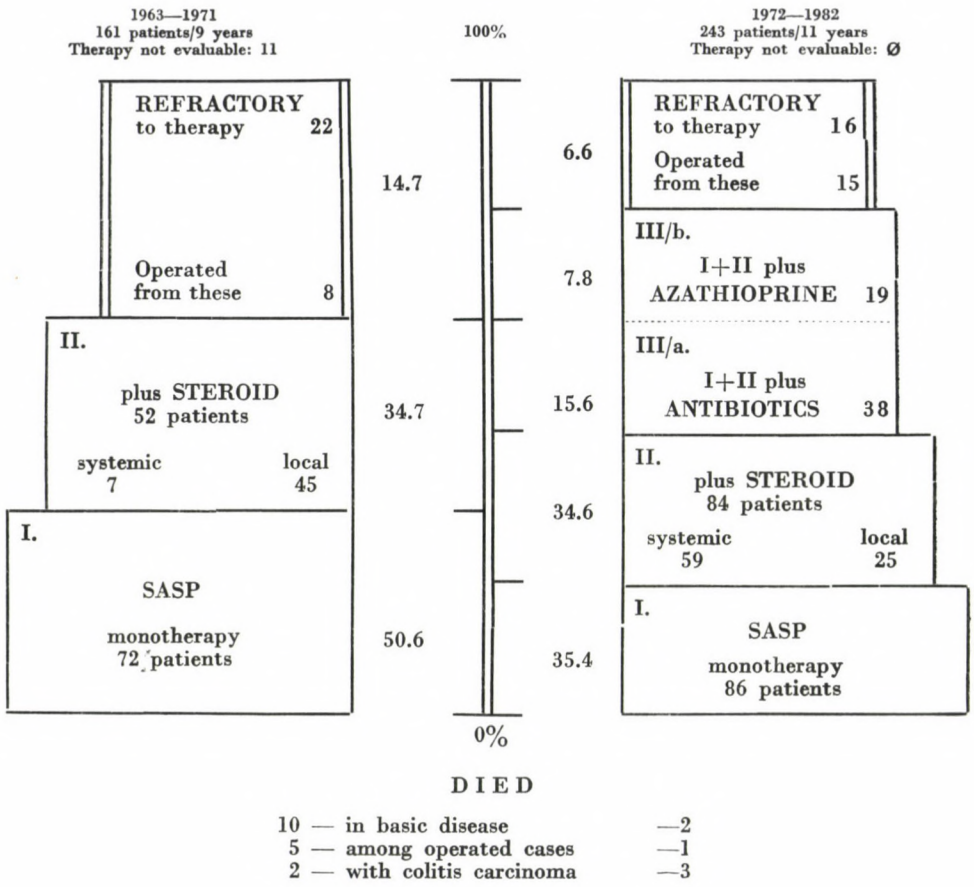


Fig. 1. Summarized therapeutic results in 404 ulcerative colitis patients

Table IV

A stepwise scheme showing the distribution of 404 patients with ulcerative colitis observed and treated between 1963—1982



DIED

10 — in basic disease	—2
5 — among operated cases	—1
2 — with colitis carcinoma	—3

Since 1972 we have been using SASP alone mostly in the moderate forms of the disease or for maintaining a remission. In cases of exacerbation we administered steroids sooner in larger doses for a short time.

The number of refractory cases was successfully decreased to approximately 6% by administering of combined antibiotics in septic forms and with azathioprine in persistent processes. When conservative therapy had no satisfactory effect, surgery was indicated sooner. As a result of these principles, since 1972 only two patients died; both were unfit for operation.

Among the fulminant ulcerative colitis cases lethality due to the basic disease was 53.8%, in the moderate or mild cases nil. The 6 colitis carcinoma

patients were mostly from the moderate group, the 5 lethal cases have already been reported [23]. Of the fulminant cases during the first therapeutical period only one out of the 12 patients recovered on drug treatment and one after surgery; 7 died during conservative therapy and 3 from among those who had been operated on. During the second period, from among 14 fulminant cases 4 recovered on drug treatment and 7 as a result of operation; 2 died during conservative therapy and 1 from among the operated patients.

In the period 1963—1982, 40 patients, 15 men and 25 women were treated with Crohn's disease. The patients' mean age at manifestation of the symptoms was 27.7 and at diagnosis 30.2 years.

The most frequent complaints were spastic abdominal pains, loss of weight, diarrhoea and subfebrility. Articular complaints and dermatological symptoms, among them SASP allergy were frequent. Bleeding occurred mostly in distal processes. Fistula and abscess could mostly be seen among the operated cases.

In altogether 23 cases the changes affected the ileocoecal region or the right part of the colon. In 3 cases the process was limited to the ileum, in 12 cases to the large bowel, and in 2 cases to the anorectal region.

In 13 cases the diagnosis was made during surgery. Operation was indicated mostly because of suspicion of appendicitis and sometimes because of symptoms of intestinal obstruction. Diagnosis in 4 cases was based on X-ray and in 15 cases on endoscopic or biopsy findings. In 8 cases diagnosis could only be made by comparing the data of different examinations (X-ray, endoscopy, biopsy and operation) and the clinical course of the disease.

In Crohn's disease, SASP alone seemed to be efficient in a single case and steroid combination in 5 cases. In 4 patients antibiotics, in 7 cases azathioprine and in 2 cases metronidazole helped in achieving a remission. We occasionally performed therapeutical trials with 5 aminosalicylic acid, SASP enema, cromoglycate, penicillamine, levamisole, etc.

Evaluation of therapeutical interventions was almost impossible because 21 of the 40 patients had to be subjected to bowel resection at one time. 21 of the 40 patients are actually well-balanced, 15 of them have chronic complaints and 4 died of septic complications.

In the patients with ileocolitis we were never forced to discontinue the basic SASP treatment owing to dose-dependent side effects. The not dose-dependent symptoms according to frequency were pruritus, eruption, febrile reaction, urticaria, methaemoglobinaemia, haemolytic anaemia, toxic hepatitis and interstitial pneumonitis. As a result of such side effects in 11/222 patients with ulcerative colitis and 5/24 patients with Crohn's disease SASP had to be discontinued. Salazopyridazine therapy often caused itches and rashes; administration of this drug had therefore to be excluded in 14 of the 182 patients with ulcerative colitis and in 7 of the 33 patients with Crohn's disease.

Discussion

In the last few decades the incidence of ulcerative colitis has not changed in the English-speaking and Scandinavian countries. The number of new cases is 4—8 per 100 000 inhabitants yearly [20, 21]. During the 20 years of our study the incidence in our region was also stationary, 3.1 per 100 000/yr. The frequency of Crohn's disease was gradually increasing and now its incidence is 2—4/100 000/yr abroad [5, 19]. The frequency in our region is now 0.58/100 000/yr but we should anticipate an increase.

The two diseases can be differentiated on the basis of clinical, endoscopic, radiologic and pathologic findings in at least 80% of the cases [11]. Differentiation in 20% is however difficult or occasionally impossible even by taking all aspects into consideration. It is because neither disease has pathognomonic findings present in every instance of the one or absent in every instance of the other disease. The type of non-specific inflammatory bowel disease therefore remained unclassified, undeterminated inspite of repeated attempts in 28 (6.9%) of the 404 patients with ulcerative colitis and in 7 (17.5%) of the 40 patients suffering from Crohn's disease.

The treatment of aspecific inflammatory bowel disease is a great problem. Estimation of the therapeutic effect is difficult because of the tendency for spontaneous remission. On the basis of our experience up to now SASP, steroids, azathioprine, metronidazole and cromoglycate are more efficient than placebo. The application of penicillamine and of aspecific immunostimulation (levamisole, BCG, transfer factor) is still in an experimental stage.

The tactics of therapy are determined by separation of ulcerative or granulomatous processes, the course of the disease, its grade of activity, anatomical localization and the complaints.

A) *Ulcerative colitis*

SASP. The orally administered drug passes into the large bowel in 85% in unchanged form, where by a bacterial effect the azo-bond is split and the drug decomposes to sulfapyridine and 5-aminosalicylic acid. In the usual relapsing type of mild or moderate ulcerative colitis SASP is significantly more effective than placebo [3, 6] but in severe attacks the result is insignificant and in the fulminant form of the disease the drug is unsatisfactory.

Sulfapyridine has a limited antibacterial effect and causes several side effects. According to some hypotheses it has the function of vehicle only [1, 12, 39]. The 5-amino-salicylic acid, considered to be the efficient component, passes with the feces in 80% unchanged and has mostly a local effect. Its inflammation decreasing cytoprotective effect is probably based on the inhibition of prostaglandin synthesis [33].

The efficacy of the drug in diarrhoea is slighter because of the short transit time and because when applying antibiotics, the SASP decomposing activity of the bacteria decreases. That is why there is a tendency for the production of new salicylate combinations.

In case of distal localization and haemorrhagic proctosigmoiditis, local administration of SASP suppository [32] and enema [9, 15] proved to be efficient. Side effects are frequent above a daily 4 g oral SASP dose, therefore, if there is no improvement in 2 or 3 weeks, steroid administration is advisable instead of increasing the dose of SASP.

In the remission stage the relapse rate of the disease without treatment during a year-long observation period was found to be around 75% [21] which could be decreased to 1/4 by continuously administering 2—3 g SASP daily [7, 21]. In order to prevent recurrences, lasting SASP therapy is necessary for a lifetime. Pregnant patients can also be treated with SASP or, if necessary, with steroid in the same way as those who are not pregnant [22, 40].

Steroids. Systemic, quickly acting steroids are given in the active stage, when the effect of SASP is unsatisfactory [14]. The optimal dose in a moderately active stage is 40 mg daily [2], in severe attacks it is 50—100 mg [37], and with fulminant symptoms it is 100 [37]—160 [13] mg daily. In the last case parenteral alimentation, adequate replacement of fluid and electrolytes, vitamins, protein and blood is an important part of therapy. If the septic symptoms do not decrease on the administration of antibiotics combined with a big dose of steroid for 5—7 days, then a surgical intervention should be indicated [37].

While the prevention of recurrences with small doses of steroid often fails, in such cases 40 mg of prednisone should be given every second day [15, 16]. The efficacy of locally applied (suppository, enema, foam) steroids in cases of proctosigmoiditis has been known for long [36]. In cases of extensive processes, oral therapy can successfully be combined with the local administration of steroid.

Azathioprine. In the acute stage its efficacy has not been proved and it does not seem to prevent recurrences [10]. In the chronic active stage, however, it decreases the steroid-demand [29]. Azathioprine administration seems to be reasonable if SASP or prednisone are inefficient or not tolerated.

The principles of drug therapy for the treatment of ulcerative colitis can be seen in Table V.

B) *Crohn's disease*

The therapy of Crohn's disease is in several aspects similar to that of ulcerative colitis. They differ in three characteristics [8].

Table V

Drug therapy of ulcerative colitis

Anatomical localization	
1. Distal process:	local organs (suppository, enema, foam)
2. Extended form:	systemic treatment
Course of disease	
1. Relapsing-remitting:	stopping of relapse (steroid + SASP) prevention of recurrence (permanent SASP)
2. Chronic continuous:	Azathioprine in case of active stage besides SASP + steroid therapy
3. Fulminant form:	large dose of steroid, antibiotics, parenteral nutrition (if necessary, acute elective operation)
4. Non classifiable:	Existence of Crohn's disease must be cleared
Grade of severity and activity	
1. Mild:	SASP 3—4 g per day
2. Moderately severe:	SASP, eventually temporary steroid 30—40 mg per day
3. Severe:	Steroid 40—100 mg per day + antibiotics
4. Fulminant:	Steroid 100—150 mg per day + antibiotics total parenteral nutrition, substitution therapy

SASP = salicylazosulfapyridine

1. In Crohn's disease the inflammatory process is of more chronic nature and affects deeper parts of the intestinal wall. The healing process is slow. Antiinflammatory treatment, including steroids needs consequently a longer period of time.

2. After surgical resection of a part of the bowel, there remains a significant tendency for recurrence.

3. While in ulcerative colitis the increased bowel loss leads to metabolic deficiency, in Crohn's disease, because of the affection of the small intestine, we must count with a damaged absorptive function.

The disease has no generally accepted therapeutical strategy. The basic principles can be gained from the present and future studies of the National Cooperative Crohn's Disease Study — U.S.A. [35] and from the Europäische Kooperative Crohn Studie [17].

SASP and/or steroid. In case of isolated colonic Crohn's disease SASP, with a process localized to the small intestine mainly steroid, and in case of ileocolitis both drugs are equally effective [16, 35]. Steroid is the more effective drug and its combination with SASP is not favoured nowadays [16, 17, 34, 35]. It was observed that patients with cobblestone bowel contours reacted much better to SASP than those without such contours [35].

Some authors consider prolonged SASP therapy beneficial in the prevention of recurrences. On the basis of controlled studies it was however stated that neither SASP nor prednisone can protect against postoperative relapses [17, 34, 35]. That is why it is now always suggested to make an attempt to prevent recurrences with the continuous administration of the drug steroid or SASP which seemed to achieve a remission [16].

Azathioprine. Reports analysing small numbers of patients showed that azathioprine had an effect on the acute phase of Crohn's disease. This was then proved in a greater controlled patient material [35]. In chronic active processes the administration of the drug's main metabolite, 6-mercaptopurine, decreased significantly the steroid-demand [27]. Therefore, for gaining time the quickly effective steroid is necessary till the slowly acting mercaptopurine has taken effect.

Considering the prevention of recurrence during a two-year-long azathioprine therapy, the recurrence rate in comparison to placebo has not changed significantly [35]. On the other hand, in patients in remission suspension of azathioprine therapy for a year significantly increased the recurrence frequency [24], and so it is probable that in a selected patient material reacting well to azathioprine, permanent administration of the drug may successfully prevent recurrences.

Metronidazole. Despite of its immunosuppressive action metronidazole seems to exert a therapeutic influence by its effect on the anaerobic flora. Although its efficacy in Crohn's disease has not been proved by the first controlled tests, a multicentric study in Sweden has shown its beneficial effect which is superior to that of SASP [28, 38]. In perianal lesions often a dramatic im-

Table VI

Drug therapy of Crohn's disease

Anatomical localisation	
small bowel	— steroid
ileocolitis	— steroid plus SASP
large bowel	— SASP, eventually metronidazole
Acute stage	
SASP 3—4 g/day	
Prednisone 0.5—1.0 mg/kg/day	
Metronidazole 800 mg/day	
Chronically active stage	
Prednisone and/or azathioprine 2 mg/kg/day	
Prevention of recurrence	
Therapy causing regression	

provement could be seen but in the case of isolated small bowel localization the efficacy was insignificant. As shown by crossed experiments, in case of a failure of SASP therapy, the change to metronidazole seems to be beneficial [38].

On the basis of the literature and our own experiences the main guidelines in the drug therapy — suggested in case of Crohn's disease — are summed up in Table VI.

Because of the wide spectrum of the clinical picture and the course of ulcerative colitis and Crohn's disease the therapeutical approach must always be considered individually.

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THE EFFECT OF D-PENICILLAMINE IN DIFFERENT EXPERIMENTAL GASTRIC ULCER MODELS IN THE RAT

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Several sulfhydryl substances were found to protect the gastric mucosa against the ulcerogenic effect of indomethacin, while being ulcerogenic in stress (restraint) ulcer. D-penicillamine showed a dose dependent antiulcerogenic effect in both gastric ulcer models. This experimental result has called attention to the two methyl groups in which the D-penicillamine molecule was different from D-cysteine. It seemed therefore that in contrast to cysteine the favourable effect of D-penicillamine in stress ulcer, was due to this structural difference.

Keywords: gastric ulcer, indomethacin, stress, sulfhydryl compounds.

Abbreviations: IND: indomethacin, STR: stress, DPA: D-penicillamine, U.I.: ulcer index.

Introduction

Szabó et al. [3] suggested that the cytoprotective action may be related to the presence of sulfhydryl-groups, since the gastric mucus contains reduced glutathione in high concentration. Bálint and Varró [2] have shown sulfhydryl substances (glutathione, cysteine, cysteamine and BAL) provided protection against the experimental gastric ulcer induced by IND while being ulcerogenic in STR (restraint) ulcer.

DPA (3,3,-dimethyl-D-cysteine) is a chelating agent and an antidote for heavy metal poisoning, capable of chemically binding various free radicals and thus to influence the physical and chemical properties of gastric mucus. Therefore, we have investigated its effect on different experimental gastric ulcer models.

Materials and methods

Ten groups of 10 female 190—220 g Wistar rats each were fasted for 24 hours prior to the experiment but were allowed water *ad libitum*. The experimental design, the ulcer models used and the mode of evaluation was published elsewhere [1, 2].

Within each group mean values ± SEM were calculated and analysed statistically using Student's *t*-test. Significant differences were assumed when the probability was less than 5%.

DPA (D-penicillamine, Serva, 31710, research grade, Heidelberg, GFR) was dissolved in water and given intraperitoneally in single doses of 10, 50, 100 and 200 mg/kg. The treat-

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ments were carried out in the STR ulcer model at 0 min, and in the 6th, 12th and 18th hour of the experimental period, while in the IND model at 0 min, and in the 2nd hour of the investigation.

Results

The experimental results are demonstrated in Fig. 1 and Table I.

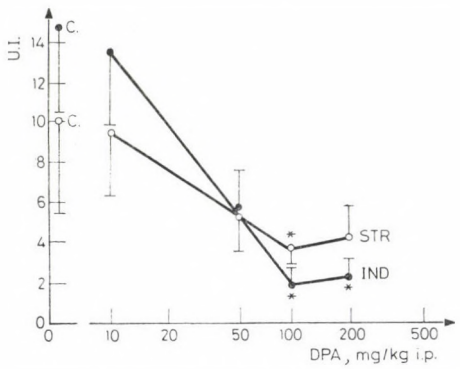

Fig. 1. Dose-response curves of D-penicillamine in different gastric ulcer models in the rat. Results are shown as mean ± S.E.M. of 10 values of each points. * = $P < 0.05$ vs Control (C). C = control; STR = stress-induced ulcers; IND = indomethacin-induced ulcers

Table I

Effect of D-Penicillamine on Experimental Gastric Ulcer

Group	Indomethacin ulcer		Stress ulcer	
	U.I. $\bar{x} \pm$ S.E.M. (Δ%)			
Control	14.4±4.39	(100.0)	10.0±4.54	(100.0)
DPA 10 mg/kg	13.5±3.81	(93.8)	9.4±3.17	(94.0)
DPA 50 mg/kg	5.6±1.74	(38.9)	5.4±1.66	(54.0)
DPA 100 mg/kg	1.9±0.52*	(13.2)	3.6±0.65*	(36.0)
DPA 200 mg/kg	2.3±0.77*	(16.0)	4.1±1.55	(41.0)

n = 10 ♀/group
* $P < 0.05$ vs Control

Discussion

From the results the following conclusions have been drawn.

1. According to our previous results [2], DPA displayed a significant antiulcerogenic property in IND-induced gastric ulceration in a dose-dependent manner.

2. In contrast to our data [2] DPA showed a strong, significant and dose-dependent antiulcerogenic effect on STR-induced gastric ulceration, too. This

calls attention to the structural difference existing between DPA and its analogue, cysteine, i.e. to the two methyl-groups in the DPA molecule. So far there are no data concerning the antiulcerogenic effect of sulfhydryl-compounds containing methyl-groups. In our opinion the methyl-groups might influence the antiulcerogenic effect of DPA in a favourable manner. Further investigations seem to be needed along this line.

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Ophthalmology

IgM PARAPROTEIN IN THE SUBRETINAL FLUID OF A PATIENT WITH RECURRENT RETINAL DETACHMENT AND WALDENSTRÖM'S MACROGLOBULINAEMIA

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IgM paraprotein was detected by immunoelectrophoresis and polyacrylamide-gel electrophoresis in the subretinal fluid (SRF) of a patient suffering from recurrent retinal detachment and Waldenström's macroglobulinaemia. Paraproteins in large quantities may inhibit fibrin formation which in turn may be an important agent in retinal reattachment and the starting point of scar formation in the subretinal space. By this case report the authors wanted to support the assumption that changes in the protein composition of SRF might explain the unsuccessful outcome in certain cases of retinal detachment when reattachment could not be achieved though the diagnosis was correct and the surgery performed correctly.

Keywords: IgM, paraprotein, subretinal fluid, retinal detachment, Waldenström's macroglobulinaemia.

Introduction

Subretinal fluid (SRF) accumulates between the photoreceptor and the pigment epithelial layers of the retina. The composition of the subretinal fluid depends on the pathogenesis and the duration of detachment [5].

In exudative detachments the SRF is highly coagulable, turbid, yellow or even brown in colour depending on the presence of inflammatory or haemorrhagic elements. If marked inflammatory changes are present, the protein content of the fluid is high, it contains immunoglobulins, fibrinogen and other serum proteins of high molecular weight in large quantities [6, 11].

In rhegmatogenous cases the SRF is at first clear and non-coagulable, its composition resembles that of the vitreous. The protein content increases with the length of the history and the changes of the protein composition in longstanding rhegmatogenous detachment are the result of irritative reaction of the choroid vessels (vasodilatation and increased permeability [6, 11, 30]).

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Fibrin formation in the SRF may be an important factor in reattachment of the retina [20]. It is considered to be the starting point of subretinal scar formation; so it probably contributes to the success of surgical procedures and promotes the spontaneous resorption of SRF in non-surgical cases [12]. Factors enhancing the change of fibrinogen to fibrin may help the process of reattachment. The inhibition of fibrin formation in the subretinal space may lead to recurrent detachments.

Waldenström's macroglobulinaemia is characterized by the accumulation of IgM paraproteins in the serum of the patient. The various symptoms of the disease are the result of disturbances in microcirculation brought about by increased blood viscosity. Extensive haemorrhages due to decreased platelet function and coagulability changes are also present in most patients. The latter are possibly caused by complex formation of IgM paraproteins with coagulation factor V [24, 28].

The ocular changes caused by Waldenström's macroglobulinaemia have recently been reviewed by Thomas et al. [27]. These changes include a dilatation and engorgement of retinal veins, retinal haemorrhages and oedema, and serous detachment of the macula and the whole retina [19].

In the present work we have studied the SRF of a patient suffering from recurrent retinal detachment and Waldenström's macroglobulinaemia, with the aim to support the assumption that changes in the protein composition of SRF may hinder reattachment of the retina.

Materials and methods

Case history

Intracapsular lens extraction was performed without loss of vitreous on both emmetropic eyes of the patient. One year after cataract operation retinal detachment developed in his right eye. Repeated unsuccessful operations (scleral resection and diathermy) were performed in another institute. The patient was admitted to our Department with a fresh detachment of two quadrants with a clearly visible tear near the equator of his left eye. Cerclage and diathermy were performed. No reattachment was observed in the postoperative period. Cerclage reoperation and the implantation of a radial plomb was the next step in surgical strategy. Though the SRF was drained, the retina remained detached after the second operation, too. Three months later the patient was operated on again, cryopexy and air insufflation were performed, without success. Beside retinal detachment the patient was suffering from diabetes mellitus, rheumatoid arthritis, polyneuropathy, Waldenström's macroglobulinaemia and constant tachyarrhythmia due to coronary sclerosis which needed special care before surgery under general anaesthesia.

Specimens

Subretinal fluid was obtained in the course of the third surgical procedure. The area around the proposed drainage site was carefully cleaned and dried. Immediately following puncture a No. 18 blunt needle on a 2 ml syringe was held over the choroidal perforation site and the SRF was aspirated as it emerged [15]. The sample was centrifuged at 500 g for 10 min. It was practically free of cells. The supernatant was stored at —20 °C until analysis within 48 hours after the operation. Serum from venous blood was drawn from the patient shortly before the surgical procedure. Tear samples were taken after the nasal instillation of 80% ethanol one hour before the operation [3].

Agar electrophoresis

Microscope slides (75×25 mm) were cleaned thoroughly and placed on a level surface. 2.0 ml of 1% w/v Difco "Agar Noble" solved in barbitone buffer (pH 8.9, 0.025 M) was run evenly over the surface of each slide. After the gel had cooled and solified 1 mm and 4 mm long troughs were cut in the middle of each slide exactly perpendicular to the axis of the electrophoretic migration. Samples of 5 μl were put in each trough with the aid of a Hamilton micropipette. Runs were carried out at 4 mA/slide for 4 hours at +4 °C [14]. A horizontal electrophoretic instrument (type 73, Reanal, Budapest, Hungary) and a DC power supply (type OE-409, Labor Műszeripari Művek, Budapest, Hungary) were used for electrophoresis in the same barbitone buffer (pH 8.9, 0.025 M). Following electrophoresis the slides were rinsed in a 1 : 1 mixture of methanol and acetic acid for 10 minutes and dried between filter papers at room temperature. Protein bands were stained in 1% aqueous solution of Amido Black 10 B for 10 minutes. Slides were put in 7% acetic acid to remove excess stain and allowed to dry.

Polyacrylamide-gel (disc) electrophoresis was performed according to the standard procedure of Ornstein [21] and Davis [9]. Gels were cast in 150 mm glass tube with an inner diameter of 5 mm. The spacer gel was 5 mm and separation gel 75 mm high. Separation gels contained 5% acrylamide monomer. TRIS-glycine buffer (pH 8.9) was used in the electrophoretic instrument. Samples of 5 μl were mixed with 50 μl 40% saccharose solution and applied directly on the top of the spacer gel. A vertical electrophoretic instrument (type 69, Reanal, Budapest, Hungary) and a DC power supply (the same as for agar electrophoresis) were used for the electrophoresis. Runs were carried out at 2 mA/tube for 30 min, and continued at 5 mA for 75 min. Gels were stained by Coomassie Brilliant Blue R 250 according to the method of Crambach et al. [7].

Immunoelectrophoresis was performed according to Scheidegger [25] with slight modification. Microscope slides (75×25 mm) were cleaned and covered with 2 ml hot buffered 1% agar (Difco "Agar Noble" solved in barbitone buffer pH 8.9, 0.025 M) in the same way as for agar electrophoresis. Two round wells were cut in the gel with a cutter (1.5 mm external diameter) in the middle of the slide at 5 mm distance from the edges. The agar plug was removed by gentle suction. The wells were filled with SRF or serum samples of 5 μl. Runs were performed in a horizontal electrophoretic instrument (the same as for agar electrophoresis). The electrophoresis was performed at 8 mA/slide for 45 minutes at +4 °C in barbitone buffer (pH 8.9, 0.025 M). At the end of the run a trough 60 mm long and 1 mm wide was cut in the gel exactly parallel to the axis of electrophoretic migration at 5 mm from the antigen wells. The trough was filled with antisera. The slides were kept in a moist chamber at room temperature for 24 hours. The excess unprecipitated protein was removed by washing in 0.9% saline solution for 2 days. Slides were dried between filter papers at room temperature. The precipitine lines were stained for 1 hour with 0.1% Amido Black 10 B dissolved in a mixture of 90.0 ml methanol and 10.0 ml acetic acid [14]. The antisera used were, anti-human polyvalent horse serum, anti-human trivalent (anti IgA, anti IgG, anti IgM) sheep serum, anti-human monovalent (anti IgM) sheep serum, anti-human lambda-specific horse serum, anti-human kappa-specific horse serum. The antisera were purchased from Human Gödöllő, Hungary.

Stains and chemicals were of analytical grade, and were purchased, if not otherwise stated, from Reanal, Budapest.

Results

The paraproteinaemia in our patient was diagnosed by agar electrophoresis. An intensive band in the region of globulins showed the presence of a paraprotein. The same characteristic band was demonstrated by electrophoresis of the SRF. The total protein concentration of SRF was approximately one tenth of the serum protein concentration therefore the composition of SRF was compared with that of 10-fold diluted normal serum (Fig. 1).

To make a precise comparison of the protein composition of the two fluids, polyacrylamide-gel electrophoresis was performed. Polyacrylamide

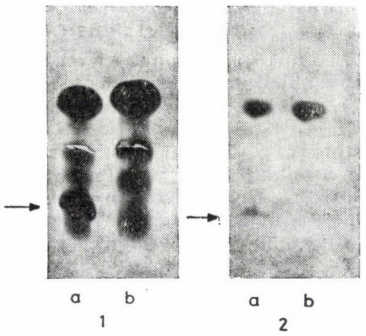


Fig. 1. Agar-gel electrophoretic patterns. *1a* serum of patient suffering from Waldenström's macroglobuliaemia; *b* normal serum; *2a* subretinal fluid of patient; *b* tenfold diluted normal serum (1% agar gel, Barbitone buffer pH 8.9, 0.025 M, stained with Amido Black). The arrows indicate the paraprotein bands

gels contained 5% acrylamide monomer, i.e. the pores of the gels were wide enough to separate large molecules like IgM and its degradation products. An intensive protein band was detected in the macroglobulin region of both the patient's serum and SRF. This macroglobulin fraction was absent from normal serum and from the tears of the patient (Fig. 2).

The paraproteins were identified by parallel immunoelectrophoresis of the patient's serum, normal serum, the patient's SRF and tenfold diluted

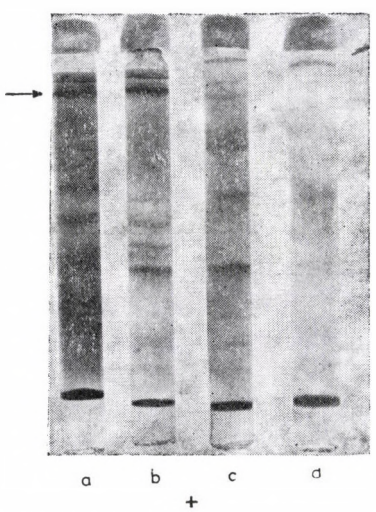
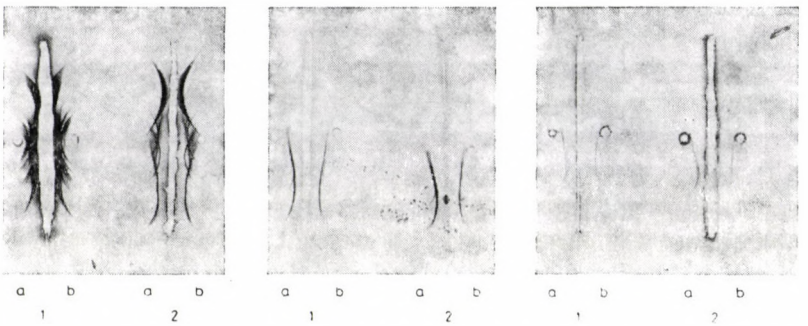


Fig. 2. Polyacrylamide-gel electrophoretic patterns. *a* 10 μl subretinal fluid of the patient suffering from Waldenström's macroglobulinaemia; *b* 10 μl tenfold diluted serum of same patient; *c* 10 μl tenfold diluted normal serum; *d* 10 μl tears of same patient (Ornstein—Davis system, 5% acrylamide-gel, TRIS-glycine buffer pH 8.9, stained by Coomassie Brilliant Blue). The arrows indicate the paraprotein bands



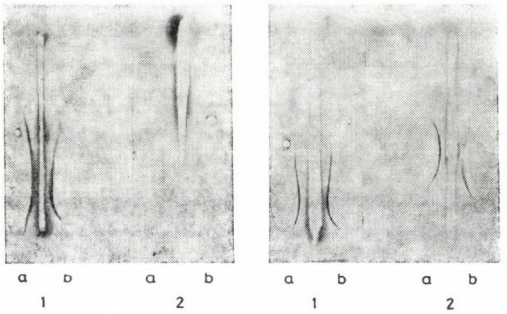
Figs 3, 4, 5. Immunoelectrophoretic patterns. *1a* serum of patient suffering from Waldenström's macroglobulinaemia; *b* normal serum; *2a* subretinal fluid of same patient and anti-human polyvalent horse serum on the first pair of slides (Fig. 3), with anti-human trivalent (anti IgA, anti IgG, anti IgM) sheep serum on the second pair of slides (Fig. 4) and with anti-human monovalent (anti IgM) sheep serum on the third pair of slides (Fig. 5). The slides were prepared according to the microimmunoelectrophoretic method of Scheidegger and stained with Amido Black

normal serum. The presence of paraprotein changed the appearance of immunoglobulin precipitin lines in the anodal part of the immunoelectrophoretic patterns and SRF (Fig. 3).

Immunoelectrophoresis was performed with trivalent immune serum, too, which contained anti IgG, anti IgM and anti IgA immunoglobulins. The longest and most intensive line in these immunoelectrophoretic patterns was the line of IgG. Another short, distinct and curved one corresponded to IgM. The third very faint precipitin line close to IgM belonged to IgA. The presence of paraproteins changed the form and place of IgM precipitin lines while the bands of other immunoglobulins were aneffected. The line of IgM in the patient's serum and SRF was highly curved and lay very close to the arc of IgG. This pattern was clearly different from the immunoglobulins of normal serum and diluted normal serum demonstrated on the other side of the slides (Fig. 4).

Similar differences were detected by immunoelectrophoresis with monovalent (anti IgM) immune serum. The precipitin lines of IgM molecules in the serum and the SRF of the patient were broad, curved and deformed, supporting the fact that both fluids contained IgM molecules of paraprotein character (Fig. 5).

Light chain analysis of the paraproteins was also performed. Immunoglobulins in the serum and SRF of our patient produced long deformed (double S shaped) precipitin lines when reacting with anti-H light chain immune serum (Fig. 6). Such deformities were not detected with anti-λ light chain antiserum (Fig. 7).



Figs 6, 7. Light-chain determination. Immunoelectrophoretic patterns. *1a* serum of patient suffering from Waldenström's macroglobulinaemia; *b* normal serum; *2a* subretinal fluid of same patient and *b* tenfold diluted normal serum. The troughs were loaded with anti-human kappa-specific horse serum on the first pair of slides (Fig. 6), and with anti-human lambda-specific horse serum on the second pair of slides (Fig. 7). The slides were prepared according to the microelectrophoretic method of Scheidegger, were stained with Amido Black

These electrophoretic patterns demonstrate that IgM paraprotein of the same type with light chain character was present in large quantities in the serum and SRF of the patient. This component was clearly different from the immunoglobulins present in normal and diluted normal serum.

Discussion

The protein content and composition of SRF was investigated by several authors in order to determine its origin in exudative and rheumatogenic cases [18, 26, 23, 13]. It is agreed that in exudative detachment the proteins are derived from choroidal vessels [4, 10, 17, 18, 22]. When there is a hole in the retina, the vitreous is supposed to penetrate through it and to lead to detachment [8, 26, 30]. In longstanding cases both factors are at play but the share of the plasma increases with the duration of detachment [5, 6, 18, 30]. In old cases the proteins disappear from the SRF with the development of choroidal atrophy [11].

The SRF protein content has been related to the outcome of surgery by several investigators. The level was significantly lower after successful than after unsuccessful operations [1, 2, 12, 29]. There was a marked increase in the protein content of SRF obtained from eyes which had undergone more than one operation [12].

Detailed examinations of SRF by immunoelectrophoretic techniques showed the presence of IgG and IgA, but IgM was absent in all specimens studied so far [6, 8, 16, 17]. These findings were usually explained by the limited semipermeability of the endothelium of the choriocapillaries and of Bruch's membrane [6, 29].

It is generally accepted that studies of the protein composition offer a more reliable indicator of choriocapillary dysfunction than do total protein estimations [6].

The significance of fibrin formation in SRF in connection with the result of detachment surgery is not yet quite clear. Orlowski [20] suggested the injection of plasma or thrombin into the subretinal space after the aspiration of the SRF. Good results were obtained by this method in a few cases. Subretinal fibrin formation may be an important factor in retinal reattachment but the process seems to be complex and must be greatly influenced by the concentration of all extrinsic pathway of coagulation. This problem probably cannot be solved simply by introduction of one or more coagulation factors into the subretinal space.

Our case may be a good example which shows the complexity of the question. Paraproteins in large quantities inhibit fibrin formation [24]. Paraprotein molecules in the SRF of the patient may have hindered reattachment of the retina in the early postoperative period. Besides, disturbances of microcirculation due to increased blood viscosity in macroglobulinaemia may have played an important role in detachment of the retina and contributed to the development of the relapses.

The conclusions drawn from the case were as follows.

1. The protein composition of SRF shows great differences depending on the type, pathogenesis and duration of the detachment, and may be affected by other diseases.

2. The protein composition of SRF may determine the result of retinal detachment operation. This may be one of the unknown causes that explain the unsuccessful outcome of certain cases of retinal detachment, when both the diagnosis and surgery were correct.

3. SRF estimations may offer useful data concerning the aetiology and pathogenesis of the detachment and the probable outcome of surgical interventions. Such data may be useful in determining the type and optimum time of operation.

4. Precise analysis of a large number of SRF-s is needed, the results must be correlated with clinical findings, and the patients must be observed for a long period before these data can be really used in the clinical care and management of patients with retinal detachment.

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Haematology

THE EFFECT OF THROMBIN ACTIVATED FACTOR XIII, THROMBIN AND PLASMIN ON THE CHEMILUMINESCENCE PRODUCED BY HUMAN NEUTROPHILS STIMULATED BY OPSONIZED ZYMOSAN (MANNOZYM^R)

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Coagulation factor XIII formed by thrombin activation from zymogen factor XIII decreases the chemiluminescence (CL) of human neutrophils stimulated by opsonized zymosan (Mannozym^R). At high concentrations, thrombin and plasmin also decreased the CL induced by opsonized zymosan. The inhibitory effect of all the three enzymes was due to their influence on the cell membrane receptors (C3b and Fc) and not to their direct effect on opsonized Mannozym^R. The potential clinical role of factor XIII, thrombin and plasma in the regulation of neutrophil functions is assumed.

Keywords: neutrophils, chemiluminescence, factor XIII, thrombin, plasmin.

Abbreviations: CL: chemiluminescence, OM: opsonized Mannozym.

Introduction

Neutrophils engaged in phagocytosis of opsonized zymosan emit a burst of light or chemiluminescence as a result of generation of reactive oxygen including superoxide anions, hydrogen peroxide, hydroxyl radicals and singlet molecular oxygen [1, 3]. Since zymosan particles bind complement components and IgG during opsonization [2], the CL inducing effect of opsonized zymosan can be mediated via C3b and Fc receptors of neutrophils.

Mannozym^R (Serobacteriological Institute "Human", Budapest) is a 0.1% suspension of zymosan (glucomannan), a cell wall derivative of Saccharomyces cerevisiae. Opsonized Mannozym^R (OM) induces mainly (about 80%) C3b and to a less extent (about 20%) Fc mediated CL in human neutrophils. In animal experiments, Mannozym^R can be used as an immunopotentiating and antitumour agent [5, 16]. Like other types of zymosan [2, 17], Manno-

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* This paper is dedicated to the memory of professor Dr. Kálmán Laki.

zym^R can activate the alternative pathway of the complement system (Sipka et al. in press) and increase the properdin level [4].

Transglutaminases catalyse isopeptide-formation resulting in cross-linked, insoluble, polymeric proteins in many cells types. The enzyme cross-links proteins by forming ε-(γ-glutamyl)-lysine cross-bridges [10]. Recently, evidence has been found of the activation of "tissue" transglutaminase during Fc mediated phagocytosis of macrophages [6, 9, 15].

The zymogen forms of transglutaminases which are present in various tissues, organs and body fluids are generally referred to as factor XIII [10]. The active enzymes formed from these zymogens by the proteolytic action of thrombin and certain other proteases are called factor XIIIa (fibrin stabilization factor) [10, 13]. The role of thrombin-activated factor XIII was proved in cross-linking of the fibrin clot and in the postejaculatory clotting of rodent seminal plasma [10].

Since the effect of tissue transglutaminase was verified in the Fc mediated activation and phagocytosis of macrophages, the present study was designed: (a) to measure the effect of thrombin-activated human factor XIII on the CL produced by human neutrophils; (b) to test the action of thrombin and plasmin on CL induction in this system; (c) to clarify the effect of activated factor XIII, thrombin and plasmin on opsonized Mannozym^R.

Materials and methods

Topostasin (human thrombin, Hoffman-La-Roche), plasmin (from human plasma, Sigma) and Fibrogammin^R (Factor XIII concentrate from human placenta, Behring-Werke AG) were used.

Opsonized Mannozym^R. Mannozym^R (1 mg) in 1 ml of Hanks' balanced salt solution (HBSS) was incubated with 1 ml of human serum at 37 °C for 30 minutes. After centrifugation at 800 *g* the pellet was washed and resuspended in 1 ml of HBSS. This suspension was used as OM.

Activation of factor XIII. 30 IU Fibrogammin^R and 30 IU thrombin were incubated together in 3 ml HBSS supplemented with 0.15 mol/l of $CaCl_2$ at 37 °C for 45 minutes. The activity of transglutaminase was tested by ^{14}C-putrescine incorporation into casein [10].

Preparation of neutrophils. Heparinized blood was diluted in 6% dextran in saline (Macrodex, 70 000 m.w., Pharmacia). The red cells were allowed to sediment for 45 min at room temperature. The leukocyte rich plasma was then layered over Ficoll (Pharmacia-Uromiro Branco Ind. Chim.) gradient and centrifuged. The pellet was treated with 0.83% ammonium chloride in order to lyse the red cells. The remaining leukocytes were neutrophils in 92—99%, viable in 99—100%. They were washed twice and resuspended in HBSS [3].

Measurement of chemiluminescence. 5×10^6 neutrophils were preincubated with thrombin, thrombin-activated factor XIII (containing equal IU-s) and plasmin at different concentration at 37 °C for 45 minutes. The photon emission of cells treated with OM and of non-treated controls was measured by an Iso-Cap/300 liquid scintillation counter (Nuclear Chicago, Searle Industries, Des Plaines, USA) in the off coincidence mode (1). The total number of photons measured three times in five minute intervals was used as a characteristic value for a given sample.

The CL induced by OM was expressed in per cents compared to the value of OM-free cell suspension, representing the basic CL, i.e. 100%. Before measuring the CL, the samples and vials were preincubated at 37 °C. The inherent cellular light and not an amplified light system was measured.

Results

Preincubation of human neutrophils with thrombin and thrombin activated factor XIII at different concentrations (in both systems the concentration of thrombin was equal), resulted in a diminished CL inducing effect of OM in both systems. But a difference could be noted in the inhibitory concentration of thrombin activated factor XIII and thrombin. Namely, thrombin activated factor XIII was a potent inhibitor of CL already at such low concentrations of thrombin (being present in the sample) at which thrombin itself (3 IU/ml) was still ineffective (Fig. 1). Thrombin also decreased the CL generated by OM, but at concentrations higher than 10 IU/ml. Both thrombin and thrombin activated factor XIII resulted in slight, non-significant changes in the "spontaneous" CL of non-stimulated cells.

Preincubation of cells with plasmin at concentrations higher than 0.7 IU/ml was also inhibitory on the production of CL in neutrophils stimuated by OM (Fig. 2).

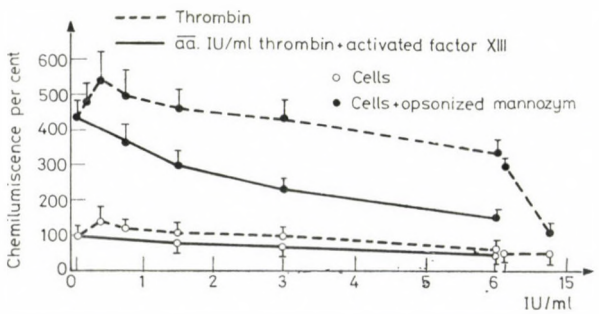

Fig. 1. Effect of human thrombin and thrombin activated factor XIII on the chemiluminescence induced by opsonized Mannozym^R in human neutrophils. (Means ± S.D., n = 4)

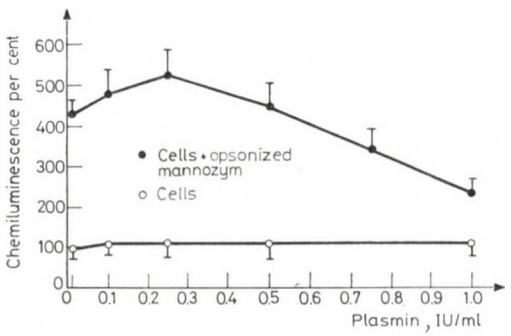

Fig. 2. Effect of human plasmin on the chemiluminescence induced by opsonized Mannozym^R in human neutrophils. (Means ± S.D., n. = 3)

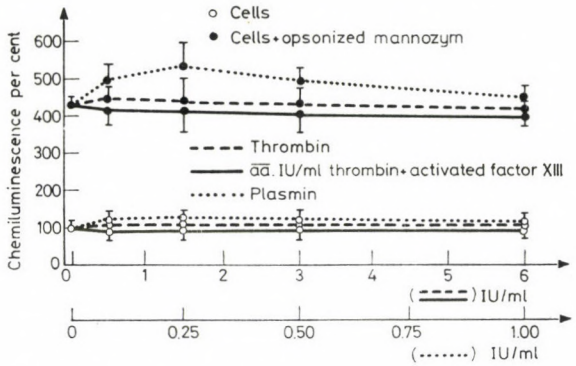

Fig. 3. Effect of preincubation of opsonized Mannozym^R with human plasmin, thrombin and thrombin activated factor XIII on the chemiluminescence induced in human neutrophils. (Means ± S.D., n = 3)

Preincubation of OM at different concentrations with thrombin, thrombin activated factor XIII and plasmin and then, the measurement of its CL inducing effects on the neutrophils (Fig. 3) did not show a significant inhibition. (In the case of plasmin treatment some slight but non significant elevation of CL induction was found.)

Discussion

Measuring the effect of thrombin activated factor XIII, thrombin and plasmin on the CL produced by OM in neutrophils, all the three enzymes decreased the phenomenon. Whereas thrombin and plasmin diminished the CL at rather high concentrations, activated factor XIII was a potent inhibitor already at such low concentrations at which thrombin itself was not yet effective. All the three enzymes acted mainly via the cell membrane receptors and not by modifying the OM.

Our findings were in accordance with previous data on the importance of transglutaminases in the regulation of cell membrane receptors [6, 8, 9, 11, 12, 14, 15], especially of Fc receptors. Since the rate of Fc mediated components in the total CL induced by OM is not more than about 20% (as mentioned in the Introduction), and thrombin activated factor XIII could result in an almost 50% decrease of total CL, it may be supposed that beside Fc receptors [6, 9] and β_2 microglobulin [8], C3b receptors can also be targets of transglutaminases on the cell surface, and that zymosan induced CL of human neutrophils can be modified not only by intracellular, but also by thrombin activated transglutaminase.

It is likely that the inhibitory effects of thrombin and plasmin on the neutrophil functions at high concentrations have in vitro, a theoretical rather

than practical importance. Although, for example, in chronic disseminated intravascular coagulation (DIC), where thrombin, thrombin activated factor XIII and plasmin are generated simultaneously in the circulation [7], these three enzymes may even worsen the clinical state by blocking the C3b and Fc mediated functions of RES or other cell types of the immune system.

Acknowledgements

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SERUM BETA-2-MICROGLOBULIN IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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Serum-beta-2-microglobulin was measured by radioimmunoassay in 25 patients with chronic lymphocellular leukaemia in stages III—IV according to the Rai classification. A significant positive correlation was found between the absolute lymphocyte count and the serum-beta-2-microglobulin level. No similar relationship was observed between the score indicative of organ infiltration and the beta-2-microglobulin value.

On the evidence of the results, the increased production of beta-2-microglobulin is attributed in the first place to the circulating lymphocytes. The assay has been found to provide a reliable indicator, suitable for the monitoring and evaluation of therapy.

Keywords: Beta-2-microglobulin — CLL — organ infiltration — absolute number of lymphocytes — therapy.

Introduction

Human beta-2-microglobulin (B2MG) is a protein of low molecular weigút, first isolated by Berggård and Bearn in 1968 (1). Its molecular weight is 11 800 dalton, its molecular radius 15 Å. Its amino-acid sequence shows a close similarity to the pattern of the light and heavy chains of IgG. Assimilarity has been found between B2MG and Bence-Jones protein as well [2, 3, 4, 5, 6].

B2MG is found on the lymphocyte surface. According to present knowledge, its synthesis takes place prevalently in the lymphocytes. On the lymphocyte surface it is present in two molecular forms, i.e. as part of the HL—A antigen complex and as a free molecule. On the úuman lymphocytes there are $3—5 \times 10^5$ B2MG molecules, roughly corresponding to fivefold of the number of HL—A antigens [5].

The biological function of this protein has yet to be clarified. Increased levels were found in malignant lymphoma and certain infections, its synthesis increases in response to the PHA-stimulation test.

The B2MG content of the human B-lymphocyte surface exceeds that of the T-lymphocyte surface by 20% [14]. The cell membrene turnover provides the most important source of free B2MG of blood-plasma and body-fluids.

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According to Amlot [3], the serum B2MG levels correlate well with the tissue infiltration of lymphoma and myeloma.

Prompted by these considerations, we have checked the serum B2MG level in a number of our patients with chronic lymphoid leukaemia, with a view to the B2MG level and the absolute lymphocyte count before and after treatment, and between the extent of tissue infiltration and the serum B2MG level-

Patients and methods

A total of 25 patients, 18 females and 7 males, of 66.6 (49—84) years average age were included in the study. At the time of study all patients were free from infections and all had normal renal functions (average se-creatinine, 88 μmol/l). Samples for B2MG estimation were taken in the morning hours. At the time of the first sampling none of the patients had yet received cytostatics. The second sample was taken after 4 weeks of combined chlorambucil-prednisolone treatment, with the exception of 3 patients who had been on combined COP-scheme, and were also checked during, as also after treatment. B2MG was measured according to the recommendations of Evrin and Wibell [9], using the Phadebas Beta-2-micro Test (normal range, 1.1—2.4 mg/l). Diagnostic criteria: absolute lymphocytosis in peripheral blood and lymphoid cell infiltration of the bone-marrow.

Clinical staging was based on the criteria proposed by Rai et al. [7]. According to this classification, 11 patients were in stage III, 14 in stage IV. For the assessment of organ infiltration the Ellegaard-score system was applied before and after treatment [8] .

The essentials of the score system are seen in Table I. Each patient scored a number according to the infiltration of bone-marrow, involvement of lymph-nodes and size of liver and spleen, evaluation was completed by linear regression analysis, using a Hewlett—Packard calculator.

The response of the absolute lymphocyte count and of the serum B2MG level to chlor ambucil-prednisolone treatment and to the COP combined cytostatic scheme were examined-

Table I

The Ellegaard-score system

	0	1	2	3
Bone-marrow	< 10% lymphocytes	10—33% lymphocytes	33—67% lymphocytes	> 67% lymphocytes
Lymph-nodes	not palpable	one in a single region	occasional lymph-nodes in different regions	multiregional large lymph-node masses
Liver-Spleen	not palpable	< 5 cm below costal arch	5—10 cm below costal arch	> 10 cm below costal arch

Table II

Effect of chlorambucil-prednisolon treatment

	Before treatment	After treatment	
Se B2MG, mg/l	4.924±0.42	2.48±0.685	$p > 0.05$
Absolute lymphocyte count: G/L	30.3±2.76	12.86±2.88	$p > 0.05$
Score	6.8±2.6	3.8±0.8	$p > 0.001$

Results

In all but 2 of the patients the serum B2MG level was increased and directly related to the absoluta lymphocyte count (Fig. 1). At 4 weeks of treatment the correlation was less close than at the beginning (Fig. 2). The response of the serum B2MG level to chlorambucil-prednisolone treatment the absolute

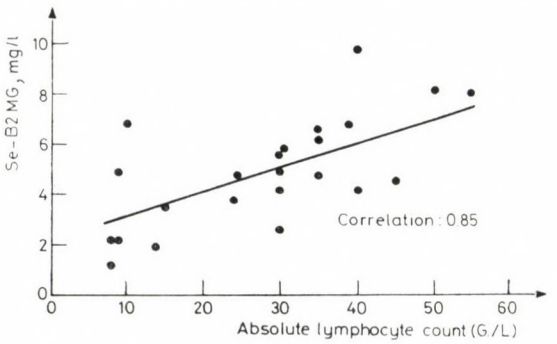

Fig. 1

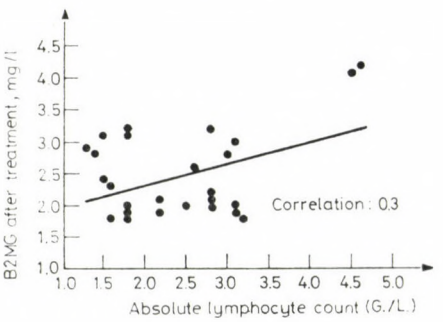

Fig. 2

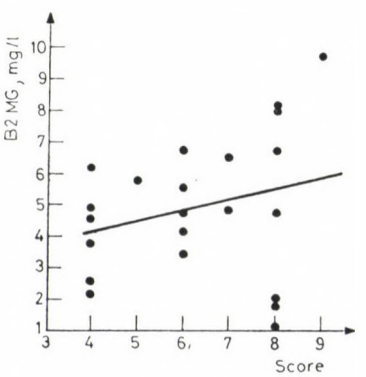

Fig. 3

lymphocyte count and of the score is represented in Fig. 3. The correlation between serum B2MG and score, as well as between absolute lymphocyte count and score, before and after treatment, is shown in Figs 4—7.

In the three patients who had been on the combined cytostatic COP-scheme the rosponse of the absolute lymphocyte count and of the B2MG levels was significant (Fig. 8).

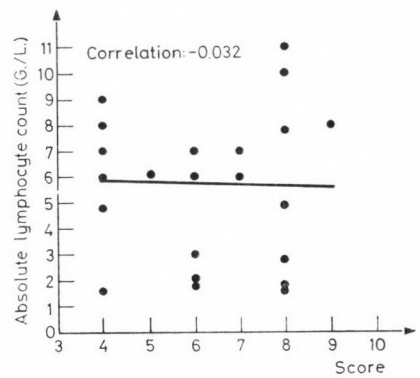

Fig. 4

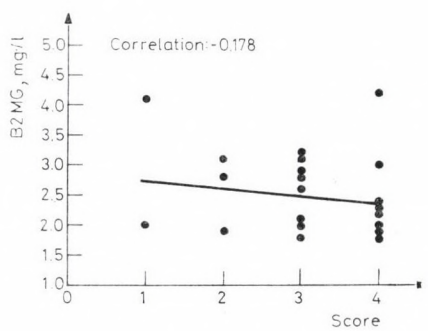

Fig. 5

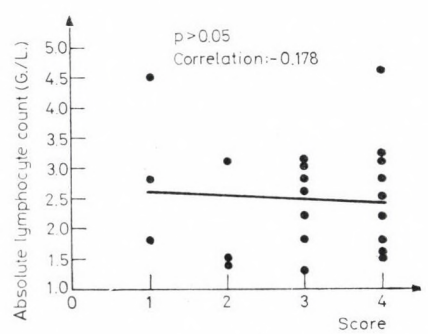

Fig. 6

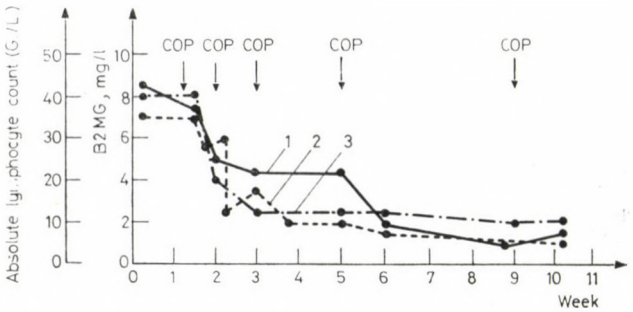

Fig. 7

Discussion

The serum B2MG level is increased in various malignant processes including myeloma and lymphoma, but high levels have been reported in infectious mononucleosis and in carcinoma. Our patients followed up and checked repeatedly revealed high B2MG levels.

We sought to identify the factor responsible for the increased B2MG level. In the untreated cases its value was chlosely related to the absolute lymphocyte count. On the other hand, therewas no positive correlation between the score reflecting organ infiltration and the B2MG level. It seems, therefore, justified to incriminate the circulating lymphocytes for the increased B2MG metabolism. It was in the first place the score indicative of organ infiltration which showed a significant decrease in response to chlorambucil-prednisolone treatment, while the decline of the absolute lymphocyte count and of the serum B2MG level was not significant. In the patients who had been on combined cytostatic COP-scheme, the serum B2MG level also showed a significant fall, together with the absolute lymphocyte count.

It has been concluded that in chronic lymphocytic leukaemia the serum B2MG level is closely related to the number of circulating lymphocytes. Measurement of serum B2MG provides a useful guide for the assessment of therapy, but the absolute lymphocyte count is of the same informative value.

The effect of chlorambucil-prednisolone treatment in the dvanced stages of chronic lymphocytic leukaemia falls short of the desired therapeutic result. On the other hand, the parameters under study showed a significant decline under the effect of the combined COP-scheme. This seems to give justification for this form of treatment in stages III—IV of chronic lymphocytic leukaemia.

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Pathophysiology

ZINK LEAD-INTERACTION IN THE RABBIT

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Subacute lead poisoning was performed in a group of rabbits by dissolving lead acetate (5 g/l) in the drinking distilled water. Another group of rabbits was left to drink the same lead acetate solution containing 0.435 g/l acetate to study the effects of zinc on lead poisoning. A third group drinking 0.435 g/l zinc acetate solution alone was studied for comparison. Intake of zinc caused a relative decrease of blood, urine and faecal lead, significant activation of the lead-inhibited erythrocyte delta-aminolaevulinic acid dehydratase (ALAD) activity and a relative decrease of urinary delta-aminolaevulinic acid (ALA) level. The analysis of several tissues for lead indicated that zinc caused biotransformation of lead from blood to some other tissues, leading to excess storage in bone, kidney and liver and less extent in the lung, brain and muscle.

Keywords: Zink lead-interaction, rabbit

Abbreviations: ALA = delta-aminolaevulinic acid ALAD = delta-aminolaevulinic acid dehydratase

Introduction

As a common contaminant of our environment, lead causes several abnormalities in living organisms of these, a widely studied subject is tbe inhibition of heme synthesis, resulting in anaemia of the microcytic hypochromic type. This anaemia suggested an alteration in the nutritional and biochemical roles of some essential micronutrients especially zinc and copper [24].

Lead poisoning in humans and animals is characterized by increased urinary excretion of delta-aminolaevulinic acid (ALA) [6, 21] preceded by inhibition of delta-aminolaevulinic acid dehydratase (ALAD), Erythrocyte-ALAD was fouud to be inhibited by lead in human subjects [4, 11] and in experimental animals [5, 10]. Although thiol compounds can activate the lead-inhiited ALAD [17], various metal ions especially zinc [22] also activate the in-

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hibited enzyme. Furthermore, zinc has been shown to be involved in the synthesis of ALAD [13].

Cerklewski and Forbes [5] found that increasing dietary zinc decreased the severity of lead toxicity in young male rats. These and another authors [9] claimed that these effects of zinc were due to its prevention of lead absorption at the intestinal level. Also, parenterally administered zinc in animals provided protection against the biological effects of acute lead poisoning [16].

The activity of erythrocyte and liver ALAD in young rats was found to be related directly to the amount of dietary zinc [2]. Furthermore, zinc at low concentration activated the lead-inhibited erythrocyte ALAD of rats but its normal activity was not restored [14]. It could be restored to normal by zinc *in vitro* but at high zinc levels the enzyme was inhibited [14]. In healthy human subjects, oral intake of zinc resulted in an increase of erythrocyte ALAD activity [1].

The excessive urinary excretion of ALA accompanying lead poisoning in lead-exposed workers was also decrased by excess intake of zinc [8]. Túis decrease of urinary ALA was observed also in rats receiving lead and zinc but it was claimed that blood lead levels were not reduced [21].

It is apparent that the effect of lead intoxication on the blood zinc level is not clear. We have therefore studied the mutual effects of zinc and lead in the rabbit. The suggestion that zinc might decrease the intestinal absorption of lead [5, 9] was also checked.

Materials and methods

Three groups of Swiss albino rabbits, 7 animals each with body weight abouts 5 kg/rabbit, were left to drink solutions of 5 g/l lead acetate (group I), 5 g lead acetate + 0.435 g zinc acetate/l (group II) and 0.435 g/l zinc acetate (group III) respectively, ad libitum for a period of 6 weeks. Before starting the experiment all animals were to drink distilled water for two weeks after which blood, urine and faecal samples were analysed for the specified parameters and the results were considered base-line values. Thereafter, poisoning was started blood, urine and faecal samples were collected weekly for 6 weeks. It was preferable to give zinc in the drinking water since all previous investigators added zinc to the diet. Furthermore, the amount of zinc used in the present study was far below the concentrations causing intoxication [17].

Heparinized blood samples were collected from the marginal ear vein by venipuncture using disposable polypropylene syringes. Parts of the samples were directly assayed for ALAD activity [25] and the remainder was prepared for lead and zinc estimation 24-hour usine samples were collected and kept without any added preservative in dark brown bottles in a refrigerator. 24-hour faecal samples were collected and prevented from contact with urine by uring a stainless steel netting put on the stainless steel cage floor ending in a polyethylene tube connected to the brown bottle in the refrigerator. Probable contamination of urine and faeces by lead or zinc, or both from the drinking solution was prevented by a special device.

Blood, urine and faeces were digested with concentrated HNO_3 "Analar" as described previously [11]. After the 6th week, the rabbits of all groups, in addition to 6 control rabbits, were killed and bones (tibia) as well as livers, kidneys, lungs, brains, hearts and the muscles were worked up. Dried bone powder was prepared by the method of Wittmers et al. [26] and the other tissues were homogenized in bisdistilled water, and then digested with concentrated HNO_3. Bone powders and tissues digests were dissolved in 0.8 M HNO_3 for the estimation of lead. Blood, urine and faeces lead and zinc as well as bone and other tissues lead were estimated

Table I

Average concentrations (±S.D.) of lead in blood, urine and faeces of rabbits drinking solutions of 5 g/l lead acetate (group I); 5 g lead acetate + 0.435 g zinc acetate/l (group II) and 0.435 g/l Zn (group III), during six weeks. The number of rabbits was 7 in each group except when otherwise stated

Test \ Duration	Base line+	1st Week	2nd Week	3rd Week	4th Week	5th Week	6th Week
Blood lead (μg/100 ml)							
Group I	15±2.0	37±2.8	78±3.5 (n = 6)	118±8.3 (n = 6)	147±10.2 (n = 6)	136±9.8 (n = 6)	146±11.6 (n = 6)
Group II	13±1.5	22±2.0	37±4.1	52±3.9	69±5.2	84±6.6	102±7.4
Group III	14±3.3	12±1.1*	9±0.6	7±1.0	5±0.3	4±0.5	2±0.1
Urine lead (mg/g creatinine)							
Group I	1.52±0.34	2.19±0.38**	2.30±0.61**	2.44±0.50 (n = 6)	2.74±0.48 (n = 5)	2.92±0.32 (n = 5)	3.21±0.36 (n = 5)
Group II	1.39±0.17	1.55±0.19*	2.10±0.17	2.19±0.15	2.34±0.09	2.48±0.17	2.86±0.14
Group III	1.44±0.41	1.39±0.36*	1.25±0.22*	1.08±0.25*	0.97±0.15*	0.98±0.17*	0.81±0.19
Faecal lead (μg/g)							
Group I	22±1.2	42±1.8	56±2.9	63±3.8 (n = 6)	78±2.9 (n = 5)	93±2.5 (n = 5)	109±6.3 (n = 5)
Group II	21±1.6	27±2.3	33±2.4	47±3.3	57±3.2	74±4.0	93±5.1
Group III	23±2.0	21±1.5*	16±0.9	11±0.7	8±1.1	5±1.3	2.1±0.5

+ Values after rabbits were kept drinking distilled water for 2 weeks before starting the experiment.
* Not significant ($P > 0.05$), ** significant ($P < 0.05$) and all other values in the Table are highly significant statistically ($P < 0.001$) as compared to the corresponding base line values.

quantitatively by a (Perkin Elmer) Flame Atomic Absorption Spectrometer model 5000. Urinary ALA [23] and creatinine (20) were also estimated. The results were analysed statistically by standard methods [19].

Results

In rabbits of groups I and II, blood lead levels were increased in the 1st week and continued increasing up to the 4th week and remained constant thereafter but the values in group II were always less then the corresponding values of group I (Table I). The blood lead level lowering effect of zinc was manifest in rabbits drinking zinc acetate solution (group III) where the blood lead level in the 6th week amounted to about one seventh the base-line value. In both groups urinary and faecal lead levels were also increased but the values in group II were always lower than the corresponding values of group I. These results indicate a storage of lead in some body tissues caused by zinc and excess storage was found in bones, kidneys and livers and less in the lungs, brains, and muscles (Table II).

Table II

Concentrations of lead (μg/g) (mean ± S.D.) in tissues of rabbits drinking distilled water (control), 5 g/l lead acetate (group I), 5 g lead acetate + 0.435 g zinc acetate/l (group II) and 0.435 g/l zinc acetate (group III) after the 6th week of the experiment. Each group includes 6 rabbits

Tissue \ Group	Control	Group I	Group II	Group III
Bone	59.6 ±5.35	159.0 ±24.59*	252.9 ±45.40*	90.0 ±11.40*
Liver	0.92±0.44	10.4 ±3.28*	21.0 ±8.75*	1.2 ±0.22$^{n.s.}$
Kidney	0.90±0.29	61.7 ±19.19*	87.7 ±17.26*	0.90 ±0.23$^{n.s.}$
Lung	1.33±0.12	4.5 ±0.91*	6.4 ±2.28*	3.4 ±1.30*
Brain	0.27±0.05	1.3 ±0.40*	1.6 ±0.22*	0.28 ±0.06$^{n.s.}$
Heart	0.21±0.09	0.17±0.02$^{n.s.}$	0.52±0.42$^{n.s.}$	0.22±0.11$^{n.s.}$
Muscle	0.05±0.03	0.07±0.04$^{n.s.}$	0.48±0.22**	0.13±0.06**

* Highly significant ($P < 0.001$).
** Significant ($P < 0.01$).
$^{n.s.}$ Not significant ($P > 0.01$).

Blood zinc levels were elevated in groups II and III during the period of study and the values in group III were higher than their corresponding values in group II. In group I (no zinc intake) blood zinc concentration was significantly ($P < 0.01$) increased in the 2nd and 3rd weeks (Table III). Urinary zinc was increased in groups II and III but faecal zinc was elevated only in group III. In group I, however, urinary zinc was higher significantly ($P < < 0.001$) decreased during the 6 week period while faecal zinc was decreased only after the 3rd week (Table III).

Blood ALAD activity was significantly inhibited in group I in the 2nd week and thereafter (Table IV). Intake of zinc with lead (group II) decreased the inhibitory effect of lead on ALAD to a great extent. Intake of zinc alone (group III) gradually activated the normal blood ALAD (Table IV).

Urinary ALA, known to be increased in lead poisoning was significantly decreased by zinc intake. Furthermore, zinc intake alone (group III) reduced the normal excretion of ALA to a minimum (Fig. 1).

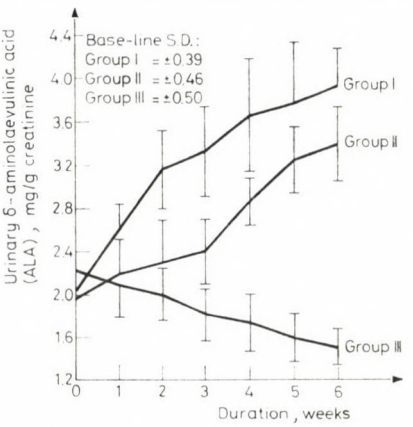

Fig. 1. Urinary ALA in rabbits drinking zinc acetate or lead acetate

Discussion

The present results have confirmed those of previous authors [1, 5, 9] who found in other animal species that oral zinc intake alleviated many toxic effects of lead. This was evident from the decrease of blood lead concentration, relative activation of lead-inhibited erythrocyte ALAD and a relative decrease of urinary ALA. It must be mentioned that although the same anion (acetate) was used for lead and zinc in the present work due to the high solubility of these salts in water and in order to prevent any precipitation of either metals, previous authors [7] found that the antagonistic effects of zinc are solely due to zinc itself and not to the anion.

The decrease in blood lead level due to zinc intake is caused by biotransformation of lead from blood to otúer tissues, especially to the bone, kidney and liver, and was not due to a prevention of lead absorption from the intestine as thought previously [5, 9]. If zinc prevented the intestinal absorption of lead then faecal lead would be raised. Actually, the faecal lead level was highly significantly ($P < 0.001$) elevated in groups I and II during the 6 week period (Table I) but the level in group II (zinc intake) was always lower than in group I. In other words, zinc may accelerate intestinal lead absorption but at the same

Table III

Average (±S.D.) concentrations of zinc in blood, urine and faeces of rabbits drinking solutions of 5 g/l lead acetate (group I); 5 g lead acetate + 0.435 g zinc acetate/l (group II) and 0.435 g/l zinc acetate (group III) during six weeks. The number of rabbits is 7 in each group except when otherwise stated

Test \ Duration	Base line+	1st Week	2nd Week	3rd Week	4th Week	5th Week	6th Week
Blood zinc (μg/100 ml)							
Group I	50±6.1	60±7.8*	68±7.0	78±4.9 (n = 6)	74±9.8 (n = 5)	76±8.7 (n = 5)	72±5.3 (n = 5)
Group II	47±5.8	68±9.0	74±4.9	80±6.6	83±7.0	84±5.8	88±7.1
Group III	53±5.0	75±4.9	79±7.0	83±5.9	90±6.0	96±6.9	106±9.9
Urine zinc (mg/g creatinine)							
Group I	1.23±0.25	0.72±0.15	0.36±0.04	0.24±0.03 (n = 6)	0.13±0.02 (n = 5)	0.06±0.02 (n = 5)	0.04±0.01 (n = 5)
Group II	0.97±0.17	0.90±0.12*	0.82±0.14*	0.62±0.12	0.44±0.07	0.35±0.03	0.28±0.08
Group III	0.85±0.26	1.36±0.45**-	2.88±0.84	5.56±1.44	11.33±2.08	21.73±4.05	53.35±7.98
Feces zinc (μg/g)							
Group I	2.36±0.64	2.17±0.60*	1.98±0.58*	1.53±0.61* (n = 6)	1.29±0.65** (n = 5)	1.15±0.61 (n = 5)	0.91±0.49 (n = 5)
Group II	2.45±0.55	2.36±0.52*	2.26±0.49*	2.14±0.45*	2.05±0.42*	1.93±0.40*	1.84±0.38*
Group III	2.25±0.68	3.88±0.89	6.03±1.07	7.80±0.91	9.29±0.47	10.44±0.99	12.37±1.17

+ Values after rabbits were kept drinking distilled water for 2 weeks before starting the experiment.

+ Not significant ($P > 0.05$), ** significant ($P < 0.05$) and all other values in the Table are highly significant statistically ($P < 0.001$) as compared to the corresponding base line values.

time accelerates its storage in some tissues and accordingly decreases the blood lead concentration, which was actually the case. This was further confirmed by the finding that in rabbits of group III (intake of zinc alone) the faecal lead level decreased gradually reaching about one tenth its baseline value at the end of the 6th week without any significant elevation of blood or urinary lead levels. Not only this but also the blood lead level was markedly decreased in this group. This means that the normally absorbed lead was directed to other body tissues by the zinc intake.

Blood, urinary and faecal zinc levels were increased in rabbits of group III (Table III) and the blood zinc level was elevated also in group II. Surprisingly the blood zinc level was further elevated in rabbits of group I (lead intake only) with a concomitant decrease of the urinary and faecal zinc levels, a finding not reported before. Zinc absorption and transfer to túe vascular system are related to the synthesis of metallothionine (in the enterocytes), the synthesis of which is proportional to the dietary intake of zinc [18]. Metallothionine is known to bind divalent metals such as copper, cadmium and possibly lead [1] and, accordingly, excessive lead absorption will interfere with the formation of the zinc-metallotúionine complex. This may explain the observed elevation of the blood zinc level in lead-poisoned rabbits. It is believed that the elevated blood zinc level is associated with increased erythrocyte protoporphyrin which has long been known to accompany lead poisoning [11]. This is consistent with

Table IV

Average (±S.D.) activities of blood δ-aminolaevulinic acid dehydratase (units+) of rabbits drinking solutions of 5 g/l lead acetate (group I), 5 g lead acetate + 0.435 g zinc acetate/l (group II) and 0.435 g/l zinc acetate (group III) during six weeks. The number of rabbits is 7 in each group except when otherwise stated

Duration	Group I	Group II	Group III
Base line++	41.9±12.78	39.9±15.01	39.5±11.15
1st Week	40.0±11.19*	37.1±14.18*	52.2± 5.62**
2nd Week	32.1± 7.60**	34.2±13.00*	61.7± 4.36
3rd Week	21.6± 8.90 (n = 6)	32.0±12.41*	74.5± 6.72
4th Week	9.8± 3.32 (n = 5)	30.1±10.14	82.5± 6.60
5th Week	7.5± 3.15 (n = 5)	24.6± 6.40**	90.8± 7.35
6th Week	5.1± 3.09	18.7± 5.07	100.3± 9.64

+ One ALAD unit is the amount of enzyme necessary to convert 1 μmol/ml/minute of 0-aminolaevulinic acid to porphobilinogen per ml of red blood cells.

++ Values after rabbits were kept drinking distilled water for 2 weeks before starting the experiment.

* Not significant ($P > 0.05$), ** significant ($P < 0.05$) and all other values in the table are highly significant ($P < 0.001$) as compared to the corresponding base line values.

the recent finding that more than half of the protoporphyrin accumulated in erythrocytes of lead-poisoned humans and animals is present in the form of zinc-protoporpúyrin complex [3, 15].

Activation of lead-inhibited ALAD by zinc is quite clear from the difference between its values in groups I and II (Table IV). In zinc-treated rabbits (group III) ALAD activity was more than twice it base-line value. This mechanism is rather obscure. However, as the enzyme is zinc-dependent and carries a large number of SH groups [17], zinc can compete with lead for the enzyme leading to its activation. The rapid increase in the activity of the enzyme from erythrocytes incubated with zinc *in vitro* [12] suggest an activation of the existing enzyme rather than de novo synthesis.

Zinc intake caused a decrease of the elevated urinary ALA in lead-poisoned rabbits (Fig. 1). Also in rabbits drinking zinc acetate alone, urinary ALA gradually decreased indicating that this effect must be secondary to the activation by zinc of ALAD. However, none of the rabbits drinking lead + + zinc acetate solution (i.e. high blood lead levels) was having a normal urinary ALA level. In other words, when the blood lead level is high, even during zinc intake, the urinary ALA level is still high. This is not consistent with results of Thawley [21] who reported normal urinary ALA in some lead-poisoned rats after orally administering excess zinc, and this placing considerable doubt on the validity of the urinary ALA screening test for determining lead exposure. It seems that the difference between the present results of Thawley [21] lies in the way of expressing ALA values in urine. Thawley expressed his results as weight ALA/24-hour urine volume while in the present study ALA was expressed in mg/g creatinine. In examining the mechanism of decreasing urinary ALA by zinc, the finding that inhibition by lead of rabbit red cell ALAD is antagonized by zinc [16] leads one to hypothesize that a lead-zinc interaction may occur in developing red cells as well as on residual ALAD present in mature red cells.

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Book reviews

E. S. Vizi, K. Magyar (Eds): *Regulation of Transmitter Function: Basic and Clinical Aspects.* Price: Hung. Ft. 690.—

Proceedings of the Fifth Meeting of European Society for Neurochemistry, held in Budapest, Hungary, 21—26 August 1984. Joint edition published by Akadémiai Kiadó, Budapest and Elsevier Science Publishers, Amsterdam 1984. XV + 571 pages, with 111 figures and 58 tables. Price: ...

The book contains 92 papers by 248 participants from 20 countries. The topics of the proceedings are arranged in thirteen sections: 1. mechanisms of neurotransmitter release, 2. transmitter receptors: molecular mechanisms and functional implications (ESN-ENA joint symposium), 3. new developments in demyelination and multiple sclerosis, 4. metabolism of neuropeptides, 5. Alzheimer's disease, 6. transmitters and neuroendocrine regulation, 7. transmitter immunocytochemistry, 9. gangliosides and neuronal plasticity, 10. benzodiazepine and GABA receptors, 11. cell surface molecules and cell interaction, 12. neurochemistry of cholinergic neurons, and 13. monoamines in invertebrates.

The proceedings are a very important source of up-to-date information on the current state of neurochemical research and its relevance to neurological and psychiatric disorders. The book contains more than one thousand full references published since 1978. The volume concludes with an author index and a subject index.

The proceedings are highly recommended to pharmacologists, biochemists, physiologists, neurologists, psychiatrics and gerontologists who are interested in the recent developments of the regulation of transmitter function.

F. Varga

The Human Environment — Past, Present and Future. Eisenbud, M. NCRP, Bethesda, MD 1983. 44 pages, 6 figures, 6 photos

This useful publication of the National Council on Radiation Protection deserves a favourable reception. Its author has managed to give a comprehensive, yet concise view of this widely involved subject, with a keen eye for the essentials, but with due consideration to the necessary details. In the introduction he enlarges on the maxim "The Past is Prologue" and approaches the problems of the interrelations between man and environment in this spirit. He might have taken a retrospective look far into the preindustrialized era, but this would have been outside the purpose of the book, since the tremendous upswing of science and technique affecting our environment dates back not more than two centuries.

The most current issues of the subject are dealt with under seven headings. First the role of growth acceleration and its involvement in the changing environment are discussed, a definition of environment being given. After the next part entitled Hierarchy of Environment, various questions of environmental pollution receive most interesting coverage. This chapter reveals some remarkable facts. For instance, according to a WHO report issued in 1964, environmental factors are somehow involved in a proportion as high as 60 to 90% of the totality of cancer cases. A further fact of adverse significance is the gradual increase in CO_2 in the atmosphere. The problems of the world's burgeoning population and of the need for energy are discussed in all their aspects. In the chapter "What is our Environmental Future?" the author faces the essential problems of the future with remarkable lucidity, keeping his predictions within the realities.

The book closes with a list of the essential references.

L. Cselkó

New Approaches to Health Education in Primary Health Care. Technical Report Series No. 690. WHO, Geneva 1983. 44 pages, 1 figure. Price: Sw. Fr. 4.—

Tis publication contains the subject matter of a meeting held by the WHO Expert Committee on the above subject in Geneva from 12 to 18 October, 1982.

Part I, by way of introduction, quotes the declaration of the Conference organized by UNICEF and WHO in Alma-Ata, USSR, 1978, that "people have the right and duty to participate individually and collectively in the planning and implementation of their health care and that "education concerning prevailing health problems and the methods of preventing and controlling them" belonged to the essential activities of primary health care. Parts 2 and 3 outline the issues and point out the concepts in the spirit of the Alma-Ata Declaration, stressing the importance of education. Part 4 gives the characteristics of the new approaches to health education in primary health care under the following headings: People-oriented health technology; Lay resources in health care; New approaches concerned with human ecology; New role for health care providers. This chapter is concise but none the less well-suited for practical implementation. Part 5 points to implications for health education practice, giving close guidelines on planning and management, ethical issues, information and communication, training, evaluation and lines of research. The book closes with Part 6, in which an excellent summary on the objectives and future tasks is found.

L. Cselkó

Research for the Reorientation of National Health Systems. Technical Report Series No. 694. WHO, Geneva 1983. 71 pages. Price: Sw. Fr. 7.—

The subject-matter of the meeting of a WHO study group held in Geneva from 27 September to 1 October, 1983, is summed up in this booklet.

Part 1 introduces the nature of health system research (HSR), its subjects and methods. Part 2 discusses the subjects of HSR in depth, giving special coverage to the aspects of society, health needs, production and distribution of resources, organizational structures of health systems, management, community participation. Part 3 deals with the performance of HSR, poses questions for research, considers, the infrastructure of organization and the implementation of research findings. Part 4 outlines the role of WHO, which is to direct, to support HSR and to correlate the activities of the member states. Part 5 sums up the conclusions and recommendations in three groups (The value of health system research; Subjects for health system

research; Performance of health system research). The tasks are set out clearly, point by point. This part will be of particular practical use. In the Summary the essentials are briefly recapitulated.

L. CSELKÓ

Evaluation of Certain Food Additives and Contaminants. Technical Report Series No. 696. WHO, Geneva 1983. 47 pages. Price: Sw. Fr. 5.—

The joint FAO/WHO Expert Committee on Food Additives held its 27th meeting in Geneva, 11—20 April, 1983, on food additives and contaminants. The subject-matter has now been published.

Part 1, the introduction, outlines the objectives of the meeting. After general considerations, Part 2 discusses various aspects of the subject (toxicological evaluation, intolerance to food additives, alterations caused by polyols, etc.) and gives a revision of some specifications. Part 3 "Comments on Specific Food Additives and Contaminants", provides detailed information on additives (antioxidants, extraction solvents, flavouring agents, food colours, preservatives, sweetening and thickening agents, etc.) and on contaminants (metals, xenobiotics, anabolic agents). In parts 4—6 specifications are revised, future tasks are outlined, recommendations to FAO and WHO are given. The publication closes with three annexes: the first gives a survey of earlier meetings held on this subject, the second lists the acceptable daily intakes, the third points to future lines of toxicologic research.

L. CSELKÓ

Mass Catering. CHARLES, R. H. G. Regional Publications, European Series No. 15. WHO, Copenhagen 1983. 70 pages, 8 figures. Price: Sw. fr. 13.—

With the development of industrialization, urbanization and tourism the mass catering systems have to meet increasing demands in industrialized, as well as in developing, countries. This book gives an authoritative, yet readable, survey on the current questions of the subject.

The brief introductory notes are followed by Chapter 1, which deals with various practical issues (bacterial and other contaminations, premises serving for catering purposes, raw material supplies, manpower, sanitary requirements, etc.) Chapter 2, General Methods of Control, covers questions of planning, licensing, management, supervision, general and personal hygiene, water problems, laboratory tests, epidemiological hazard analysis critical control point (HACCP). Chapter 3 is on new technologies (cook-freeze and cook-chill, slow cookers, microwave cooking, convenience foods, electronic thermometers, anaerobic packing). Chapter 4 deals with training. Chapter 5 discusses the pertaining problems of institutional and welfare catering, including hospitals, old people's homes and clubs, meals-on-wheels. In Chapters 6—8 catering in canteens of factories and other commercial establishments, open-air catering (disasters, festivals, tourist hotels, holiday camps) are considered. Chapters 9—10 provide information of particular interest on travel catering (aeroplanes, ships, other vehicles). Chapter 11 is on banqueting. The book closes with a summary in Chapter 12.

L. CSELKÓ

Planning the Finances of the Health Sector. MACH, E. P., ABEL-SMITH, B. WHO, Geneva 1983. 124 pages with 1 figure and 21 tables. Price: Sw. Fr. 14.—

Many countries, particularly the developing ones, are seeking to orientate their health services towards a more equitable and efficient utilization of resources. A detailed analysis of the financing of health services is an important step in such an undertaking.

This manual sets out a methodology for carrying out an analysis, suggesting ways of collecting and organizing data on expenditure and financial sources. It also suggests how this information might be utilized in policy formulation, to make a master plan for the future use of all financial and material resources. Particular attention is paid to primary health care in view of its high priority in current health policies. A series of tables presents models that provide an analytical framework for national planning, and summarizing tables have been devised for the use of policymakers.

The manual is aimed at planners, economists, statisticians, accountants and researchers in the health and health-related sectors in developing countries, and at the staff of international and bilateral agencies concerned with development aid.

By this manual comprizing 8 chapters, a list of 30 references and 2 annexes, the author has provided workers of developing countries with an excellent guide, more of practical than of theoretical orientation.

L. CSELKÓ

Primary Health Care — The Chinese Experience. WHO, Geneva 1983. 105 pages, 9 tables, 12 figures, 17 photos. Price: Sw. Fr. 14.—

An Inter-regional Seminar on Primary Health Care took place in China, from 13 to 26 June 1982. Jointly organized and financed by UNDP, NICEF, the World Bank, and WHO, with the support of the Ministry of Public Health of the People's Republic, it was held at the WHO Collaborative Centre for Primary Health Care in Yexian County, Shandong Province.

The objectives of the seminar were:

a) to explore some aspects of experience in primary health care in China, with particular attention to

— the three-level network of the health care system
— the people's involvement in, and management of, health care
— health manpower development
— financing of health care,

b) to draw conclusions applicable to the development of primary health care in other countries.

The book comprizes 4 chapters and an appendix. Chapter 1 introduces the subject of the seminar and provides detailed information on China. Chapter 2 "An Introduction to Rural Health Services in China", deals with the health situation in the rural areas. This is the most interesting part of the book, in view of the fact that more than 80% of the Chinese population of more than a thousand millions, live under rural conditions. The length of Chapter 3, "Aspects of Primary Health Care in China" is in proportion to the importance of its subject. The following subdivisions are of particular interest: The three-level network of the health care system; The people's involvement in and management of, health care; Health manpower development; Financing rural health care. Information provided by these sections brings the health situation prevailing in China into due prominence. Chapter 4, presenting the conclusions of the seminar, opens with the following general considerations:

China has demonstrated a tremendous political commitment to the task of changing the quality of life of all its people and especially of the rural population. Health goals have been given very high priority. This political commitment permeates all levels of government and all social and mass organizations ensuring sustained popular support.

The reorganization of the country's economic and social structure, and in particular the high level of decentralization has permitted the integration of the health sector with all aspects of economic and social development and has facilitated the people's involvement in the financing as well as the management of health care.

Concerted action in many sectors has contributed to raising the level of health of the people. Sufficient increase in income and its equitable distribution to permit minimally adequate shelter, clothing and, above all, essential food at affordable prices, the expansion of literary and mass education (particularly primary education), the provision of public services such as water supplies and transport, the policies and programmes related to family planning, etc., have all contributed to this improvement of the health status.

Perhaps the most important factor in the development of the health care system has been the participation of the people in the provision of health services, in the management of the system, and in mass campaigns. The people have contributed to the integration and better coordination of health programmes and to intersectoral collaboration of health programmes and to intersectoral collaboration at all levels.

Every step in development of the Chinese health care system in the past—starting with mass mobilization for prevention, followed by the development of cooperative health centres, the emergence of the "barefoot doctor", the combined use of traditional Chinese medicine and western medicine, the development of the commune and brigade network with its cooperative medical insurance schemes and of the whole supportive health care network at higher levels, etc., is a concrete and living expression of what constitutes appropriate technology. In this chapter some further aspects of primary health care possibilities and tasks involved by participation of the population, utilization of financial resources, development of manpower, are also discussed. The Annex contains numerical and other data, supported by maps.

The book contains such an immense wealth of information that any attempt at condensing it into a review, even in the form of referring to its bare essentials, would be futile.

L. Cselkó

Effects of Nuclear War on Healths and Health Services. WHO, Geneva 1984. 178 pages, 37 tables, 21 figures. Price: Sw. Fr. 20.—

The 1981 WHO Assembly adopted the resolution that, in the spirit of the WHO Constitution that the health of all peoples is fundamental to the attainment of peace and security and in view of the involvement of physicians and other health workers in the tasks connected with this goal, an International WHO Committee of Experts met on 14—18 April 1982, 2—4 November 1982 and 10—11 February 1983, in order to discuss the health problems of nuclear war. This book presents a global report of these meetings. It opens with the following conclusions emerging from the reports and discussions:

1. "Conventional" wars are continually becoming more destructive. However, the introduction of nuclear weapons has added totally new dimensions to warfare.

2. A single thermonuclear bomb can have an explosive power of a million times the largest conventional bombs and the present stockpiles of nuclear weapons have an explosive power thousands of times greater than all the explosives detonated during the Second World War. In addition to the effects of blast and heat, the radiation and nuclear fallout of a nuclear explosion can have devastating effects, both immediate and long-term.

3. The Committee has considered three possible scenarios:

1. The detonation of a 1-megaton bomb over a large city would kill more than 1.5 million people and injure as many.

2. "Limited" nuclear war with smaller tactical nuclear weapons totalling 20 megatons, aimed at military targets in a relatively densely populated area, would exact a toll of about 9 million dead and seriously injured, of whom more than 8 million would be civilians.

3.An all-out nuclear war using at least half of the estimated present stockpiles of nuclear weapons (an approximate total of 10 000 megatons) would result in more than 1000 million injured people.

4. It is obvious that no health service in any area of the world would be capable of dealing adequately with the hundreds of thousands of people seriously injured by blast, heat or radiation from even a single 1-megaton bomb. Even, the death and disability that could result from an accidental explosion of one bomb from among the enormous stockpiles of weapons could overwhelm national medical resources.

5. It is difficult to comprehend the catastrophic consequences and the human suffering that would result from the effects of nuclear explosions in the second and third scenarios that are considered. Whatever remained of the medical services in the world could not alleviate the disaster in any significant way.

6. To the immediate catastrophe must be added the long-term effects on the environment. Famine and diseases would be widespread, and social and economic systems around the world would be totally disrupted.

7. Therefore, the only approach to the treatment of the health effects of nuclear explosions is primary prevention of such explosions, that is, the prevention of atomic war.

8. It is not for the Committee to outline the political steps by which this threat can be removed or the preventive theraphy to be implemented.

9. However, WHO can make important contributions to this process by systematically distributing information on the health consequences of atomic warfare and by continuing and expanding international cooperation in the field of health.

Part I contains introductory comments. Part II, "Physical Characteristics of Nuclear Explosions and their Effects" gives insight into various aspects of the subject, including phenomena occurring when nuclear weapons are exploded, followed by details, such as effects of size of bombs and height of explosion, electromagnetic pulse, effect of nuclear detonation on human beings (blast wave, thermal wave, initial radiation, local and global radioactive fallout, effects of radiation on the body. In the same chapter three predicted scenarios of a nuclear war are presented, viz.

Scenario 1. London was chosen as an example. In both varieties a bomb of 1 Mt (megaton) was supposed to detonate, the first in the altitude of Hiroshima, i.e. at 580 m, the second in an altitude of 2500 m. The casualties for the two altitudes are largely identical, i.e. 1 800 000 dead, 1 700 000 injured for low altitude, 1 600 000 dead, 1 600 000 injured for high altitude. In sum 25% of the population would be killed and another 25% wounded.

Scenario 2. Local use of nuclear weapons. Here military targets in Central Europe are assumed to be attacked with tactical nuclear weapons of 100—200 kt (kiloton). In this case the explosive power of the tactical nuclear weapons would add up to 20 Mt which, according to the calculations, would result in 9 million dead and the same number of severe injuries and about the same number of less severe injuries.

Scenario 3, all-out nuclear war. In this case 10 000 Mt nuclear bombs would be exploded all over the world, 90% in Europe, Asia and North America, 10% in Africa, Latin Africa, Latin America and Oceania. The result of such a war would be 1 150 000 deaths and 1 100 000 000 injuries. Half of the world's entire population would be immediate victims.

In Part III, on the management of casualties, the scope of the problem is outlined and guidelines on treatment are offered. For this part, as well as for Part IV, on the short-term and long-term effects of nuclear war, supplementary information is provided in the Annexes. Part VI, Conclusions, is followed by a glossary and a selected bibliography.

This information is condensed into the first 37 pages of the book. The remaining, considerably larger, part is occupied by 9 annexes which contain a vast body of highly important factual information on thermonuclear weapons and nuclear war, including the short-term and long-term effects of thermonuclear weapons on individuals and health services, the effects of medium- and long-range weapons, carcinogenic, teratogenic, genetic involvements, and the possibilities of the health services in the case of a nuclear war. In Annexes 4 and 5 we find detailed information on the health effects on individuals and health services of the Hiroshima and Nagasaki bombs.

In view of its high significance the book deserves to be translated into various languages, so as to be made accessible to physicians and health workers all over the world.

L. Cselkó

Apartheid and Health. WHO, Geneva 1983. 258 pages, 29 tables, 2 figures. Price: Sw. Fr. 29.—

This book claims particular interest. Its subject, dealt with in two parts, is very timely. By taking a look at the health problems posed by apartheid, it gives plenti of food for thought and discloses appalling facts.

Part I. *Report of an international conference held at Brazzaville, People's Republic of the Congo, 6—20 November, 1981*

Chapter 1, serving as introduction, is followed by the opening statements from the following participants: C. A. A. Quenum E, Regional Director for Africa, WHO; A. Nzo, Secretary-General of the African National Congress; I. N. Pokela, Chairman of the Pan Africanist Congress of Azania; I. Idongo, Secretary of Health, South West Africa People's Organization; and H. Mahler, Director General, WHO. Chapter 3 is centred on the main themes of the Conference which may be listed without further commentary: Heath or Apartheid? Analysis of the child care delivery system in apartheid South Africa; Apartheid and maternal and child health; Apartheid and worker's health; Apartheid and mental health. The disastrous effects of racial discrimination on the health situation of the black population are highlighted by all statements and comments. The recommendations in Chapter 4 span a wide range and extend to direction, coordination and management, health system infrastructure as well as technology. In Chapter 5, the main lines of the strategy for "health for all by the year 2000" in the African Region and action against racial discrimination are pointed out. This is followed by a detailed plan of action. Its structure is consistent with the main lines of WHO-s general programmes. Chapter 6 contains the Brazzaville Declaration in its full text. Section I closes with two annexes connected with the programme of the Conference.

Part II is an analytical report to the Conference on the health implications of racial discrimination and social inequality. Explanatory comments upon some definitions are given. Chapters 1—2 give information on the nature of apartheid, backed by statistical figures and the origins of South African society, and its health care system. Chapter 3 deals with the living conditions and pattern of disease; it presents general and special morbidity and mortality figures reflecting a sadly disadvantageous situation of the black versus white population. Some appalling examples: there were 16-times more deaths due to infectious diseases among the coloured than among the white population. Tuberculosis accounted for 1/3 of these deaths. The death rate of infectious diseases is 12-times as high in the coloured than in the white

population of advanced age-groups, and tuberculosis is responsible for 80% of these deaths. The figures of maternal and child health fare in no way better. Other diseases also show a striking prevalence in the coloured population. Similarly alarming figures emerge from Chapter 4 which deals with the extent and effect of malnutrition. This is also true for Chapters 5—6, discussing the impact of apartheid on psychosocial development and occupational health and diseases. The aspects of the situation outlined above are reflected in the health services dealt with in Chapter 7, giving a survey of the politics of health care. In conclusion, the situation of disease and health care in South Africa is viewed in the light of the Constitution of the World Health Organization.

L. Cselkó

R. H. Bannermann, J. Burton, Ch'en Wen-Chieh: *Traditional Medicine and Health Care Coverage.* WHO, Geneva 1983. 342 pages, 4 tables, 1 figure, 22 photos. Price: Sw. Fr. 35.—

This book has the invaluable merit of providing clear, comprehensive and thoroughgoing information in 28 well-arranged chapters on the various types of traditional medicine which are still in practice on a larger or lesser scale in various parts of the world.

Part I takes a general look at the systems and practices in traditional medicine. Chapter 1 gives an excellent introduction into the new discipline of "ethnomedicine". In Chapter 2, which deals with traditional medicine in Africa, the role of the traditional healers and the various practices of traditional medicine are given due consideration. Chapter 3, on traditional medicine in Latin America, centres on two subjects: humoral theory and therapy, in which context the meaning of "Hot" and "Cold" are explained, and on spiritism. Chapter 4 explains the various aspects of Ayuverdic medicine, which has spread from the Indian subcontinent. The doctrine of medical astrology is also given consideration. Chapter 5 provides information on the basis concepts and practice of the Unani system of health, referred to also as Graeco-Arab, or Arab medicine. This system had its roots in the Arab civilization and its practice has been revived in the Indo-Pakistan subcontinent. Chapter 6 takes a look at the past and present of traditional Chinese medicine and views it in the framework of the present health system. Acupuncture and moxibustion are dealt with, and the lines of research in acupuncture are pointed out in Chapter 7. Chapter 8, on treatment of fractures and soft tissue injuries by integrated methods of traditional Chinese and Western Medicine claims particular interest. Chapter 9 reviews the history, types and organization of modern allopathic medicine and public health, and advocates a closer integration of traditional medicine with allopathy. In Chapters 10—13 we find information of exemplary objectivity on homeopathy, naturopathy, divination and Exorcism, and Hypnosis. Chapter 14 is on practices of yoga and meditation. Chapter 15 describes the practices of traditional midwifery and contraception, pointing to lines of research and to ways of integration with modern health services. In Chapter 16 selected individual therapies such as antroposophical medicine, autogenic training, breathing, biofeedback, hydrotherapy, etc., are considered.

Part II deals with herbal medicines and herbal pharmacopoeias. Chapter 17 covers the endangered plants used in traditional medicine, providing a list of these plants. Chapter 18 is on the NAPRALERT system. This is a computerized data base on the chemistry and pharmacology of natural products and has been given the acronym NAPRALERT (Natural Products ALERT) Chapter 19 takes a look at the situaation of phytopharmacology and phytotherapy, medical plants, plant drugs and utilization of local plant resources in primary health care.

Part III, in Chapters 20 to 25, gives a profile of the traditional practices in the WHO regions, i.e. in the six principal regions, with close details on the situation in the individual countries.

Part IV covers the organizational and legal aspects of traditional medicine. In Chapter 26, the organizational tasks are outlined. In Chapter 27, legal aspects, patterns of legislation concerning traditional medicine and policy options in regulating the practice of traditional medicine are discussed in close detail. Chapter 29 gives a brief but none the less interesting, closing review of the place of traditional medicine in primary health care.

L. CSELKÓ

Smoking Control Strategies in Developing Countries. Technical Report Series No. 695. WHO, Geneva 1983. 92 pages, 1 figure. Price: Sw. Fr. 8.—

The meeting held by the WHO Expert Committee on Smoking Control Strategies in Developing Countries in Geneva from 22 to 27 November is reported in this publication.

Part 1, the Introduction, gives an exhaustive explanation of the reasons for discussing the smoking problem of the developing countries at a high level. Though the book is primarily addressed to developing countries, the problems and tasks it deals with also apply to developed countries.

Part 2, entitled The rationale for smoking control, contains a wealth of information on the epidemiological and economical aspects, the prevalence of smoking including the traditional and other tobacco habits and their high risks, as also on the constituents of smoke. The growth of tobacco industry is seen against the background of declining food consumption in some of the developing countries, thus raising bluntly the alternative, "food or tobacco". It has been further found that smokers have higher annual demands on medical services than non-smokers. On the evidence of a WHO-study, in Canada, the health care costs connected with smoking could be estimated for a certain time interval at US S 24 000 million, to which another 15 000 million due to loss of productivity and absenteeism from work has to be added. In the framework of Part 3, entitled Smoking Control in Developing Countries, the objectives are defined, the control programmes outlined, guidelines for data-collection are affered, public information and public education programmes are provided, the role of media being emphasized. The necessity for and the chances of, restrictive measures and legislation are pointed out. The activities serving the primary goal, the cessation of smoking are summed up. Part 4 points to the lines of research. In Part 5, on international action, the activities of WHO as well as of other organizations are considered. Part 6 sums up the recommendations. The book closes with a list of 118 references. There are two annexes. The first recapitulates the recommendations of previous WHO expert committees on smoking. The second sums up the countering arguments against the control of smoking.

L. CSELKÓ

M. J. SUESS, J. W. HUISMANS: *Management of Hazardous Waste.* WHO Regional Publications, European Series No. 14. WHO, Copenhagen 1983. 101 pages, with 4 tables, 2 figures. Price: Sw. Fr. 10.—

"During the past decade, the public has become increasingly aware of one of the major consequences of industrial development — the quantity and diversity of the hazardous waste it generates. At the same time, awareness has been growing that certain disposal methods used for such waste may pose risks to human health and the quality of environment. Several countries have made efforts to develop effective technologies and administrative procedures for hazardous waste management."

The brief introduction of this very timely book is followed by policy guidelines in Chapter 1, in which general principles and some specific aspects are pointed out. Chapter 2, which defines the problem and sets criteria, falls into the following subdivisions: Criteria for identifying hazardous waste; Exclusive and inclusive list of hazardous waste; Waste hazardous in only part of the management cycle; Examples of hazardous waste; Other waste-arisings. Chapter 3 formulates the legal and administrative requirements. In Chapter 4 the basic lines of planning are set out. Chapter 5 deals with the tasks of collection, transport and storage, and Chapter 6 with those of management, treatment and disposal. Chapter 7 gives recommendations for enforcement, Chapter 8 offers guidelines for transfrontier transport. Annex 1 gives the essential references. Annex 2 lists the non-hazardous wastes, Annex 3 gives examples of hazardous waste. In Annex 4 there is a list of the types of solution and waste-water from metal finishing. In Annex 5 we find the conclusions and recommendations of the Working Group on Guidelines for the Control of Toxic and other Hazardous Chemical Waste, Garmisch-Partenkirchen, 17—20 March, 1981.

One of the principal merits of the book is its clear, logical arrangement. It provides invaluable guidelines for the management of hazardous waste.

L. Cselkó

Recommended Health-based Limits in Occupational Exposure to Pesticides. Technical Report Series No. 677. WHO, Geneva 1982. 110 pages, 16 tables, 1 figure. Price: Sw. Fr. 8.—

This is a report of a meeting of the WHO Study Group held in Geneva from 15 to 22 June, 1981.

In Part 1 serving as introduction, the purpose of the report is outlined and the procedures, evaluation of the studies, the relationships of exposure-effect and exposure-response are discussed, and conversion factors applied to express the concentrations of the vapours of the substances in the air in terms of milligramms per cubic metre are given. Experts are invited to provide WHO with any additional data that may serve for re-evaluation of the recommended health-based exposure-limits of the four substances malathion, carbaril, BHC (Lindane) and dinitro-o-cresol, which form the actual subject of the report. Parts 2—5 cover these four pesticides in close detail, discussing invariably in the same order their properties, uses, health hazards, assessment of exposure, relationship between exposure and effect, conclusions, recommendations, research possibilities. These four chapters contain essential information for specialists engaged in this field. Part 6 gives recommendations for research, emphasising the health of agricultural workers, the need for epidemiological data, animal experiments, metabolic studies, proper surveillance and the special problems of developing countries.

Each chapter is exhaustively referenced. The text is backed by clear, instructive tables.

L. Cselkó

Hormonal Biology of Endometrial Cancer. Edited by G. S. Richardsen and D. T. MacLaughlin. Geneva 1978. 187 pages.

In this volume the results of research were discussed at a conference held under the sponsorship of UICC in Geneva between 6 and 10 February, 1978, and the conclusions have been integrated by the editors into a coherent whole. The topics of the conference were strictly confined to the hormonal aspects of endometrial hyperplasia and cancer.

It begins with introductory notes on the risk factors of endometrial cancer, including the prevalence of oestrogenic effects, the possible role of androgens, and on the progression of endometrial hyperplasia, as well as of the malignant processes of epithelial origin. It deals exhaustively with the intracytoplasmic proteins of high specific hormone-binding affinity, with the quantitative changes in the receptors and with oestradiol-17-beta dehydrogenase induction, considering also the molecular mechanism of action of oestrogen and progesterone and the formation of potentially steroid-specific products.

The quantitative changes of the receptors and their characteristics in case of hyperplasia and carcinoma are described in detail. It is pointed out that oestrogenic activity, unless interrupted by progesterone, will result in hyperplasia or carcinoma. Progesterone inhibits oestrogenic activity by blocking the function of the receptor system on the one hand, and by inducing the activity of the enzyme oestradiol-dehydrogenase, on the other. This effect may result in a rapid regression, or a complete disappearance, of hyperplasias, dysplasias, even of carcinomas, particularly of those of differentiated type. It is also emphasized that the gestagens, despite their benefits, provide no complete solution to the therapeutic problems of endometrial cancer.

Cervical carcinoma responds to progesterone in one third of the cases and the responsive tumours are prevalently of well differentiated types. Undifferentiated tumours respond in no more than 15% of the cases. For this reason additional use of progesterone is advocated by the authors in all cases in which chemotherapy is justified.

The trends of future research are pointed out in the closing chapter.

The monograph gives an excellent summary of the successive stages of the process resulting in endometrial cancer, covering also the hormonal aspects of this tumour, as also the results of, prevalently biochemical, research attained until 1978 in this field. It commends itself by its clear arrangement, its easy and readable language, its exhaustive references, as well as by the large number of its instructive tables, to research workers and to all those seeking information on the subject.

S. CSÖMÖR

Second Cancer in Relation to Radiation Treatment for Cervical Cancer. Editors N. E. DAY, J. D. BOICE JR. Lyon 1983. Price: £ 17.50

This volume published by an international working party of WHO on the irradiation therapy of cervical carcinoma is the 52nd number of the serial publications of IARC (International Agency for Research on Cancer).

This international study of the radiation therapy of cervix carcinoma is centred on irradiation carcinogenesis. The aim of the programme is to provide quantitative information of maximum accuracy on the prevalence of new, dose-related carcinomas formed in other organs, thus contributing to the understanding of irradiation carcinogenesis. With this objective a group of patients a number of whom had been exposed to known irradiation doses were followed up in order to find out whether new primary tumours had formed in other organs, and if so at which sites and after which therapeutic schemes. The observations were supported by dosimetry, pathologic evaluation and chromosomal studies. The findings of new tumours were checked against the predicted prevalence of the respective tumours for the same age-groups.

The book comprizes an introduction, a list of the participants in the study, description of methods, data of some cancer registers providing the basis for the study, radiation exposure of other organs involved by irradiation of the uterine cervix, a summary and appendix with comprehensive data in a tabulated form.

The data of 15 cancer registers have been studied separately and also on a comparative basis. In consideration of the differences between the various forms of treatment, the patients were divided into two groups according to whether or not they had received radiotherapy. In view of the inconsistency of staging during the long follow-up period, only "invasive" and "non-invasive" groups were distinguished. The time of production of the second, new primary carcinoma was correlated to the time of diagnosis of the cervical carcinoma. If no second carcinoma had been formed, the time of death or of the last follow-up was considered. The data listed in a tabulated form of consistent arrangement form the bulk of the book. A historical review of the recording systems and of data-collecting accompanying the tables makes the interpretation of bare data easier and the book more readable.

From the chapter providing a comprehensive review it emerges that the data of more than 180 000 patients with cervical cancer have been analysed. The process of 91 000 had attained the invasive stage and 87 000 of these patients had received radiotherapy. Carcinoma in situ was found in 84 000 cases. The years of follow-up of the irradiated patients totalled approximately 625 000, and 10 years after diagnosis of this tumour the total figure was 180 000 years. The average period of follow-up was 7.1 years. In the patient material studied the production of a second carcinoma was observed in 3 324 of the irradiated cases, as against the predicted number of 3 063 in an age-matched population. It was a tumour of the lung, of genital regions, of bladder and rectum which accounted in the first place for the difference, but the differences for the occurrence of tumours of oral cavity, oesophagus, small intestine, pancreas, for mesenchymal tumours and for acute and chronic leukaemia were also significant. It was primarily breast cancer which had an incidence lower than predicted. Pulmonary cancer was of equally high incidence in the non-irradiated and in the irradiated cases, as also in the case of carcinoma in situ. Interpretation of the figures requires caution, since in the production of pulmonary carcinoma other carcinogenic factors (e.g. smoking) may also be involved.

It is concluded from the results that the risk of a second carcinoma involved by irradiation of the cervic scarcely exceeds the predicted incidence of the respective tumours; in other words, the high radiation exposure in cervical cancer scarcely adds to the hazard of a second carcinoma. The sites of production of a new carcinoma, as well as the time elapsed since the irradiation, might be compatible with a possible role of earlier radiotherapy in individual cases. It is emphasized, however, that exposure to moderate or major radiation doses (over 100 rad) carries an increased hazard of a second carcinoma, particularly of bladder, rectum, bones, connective tissue, uterine corpus, ovaries, small intestine, kidney, as also of multiple myeloma. Ovarian irradiations have reduced the risk of breast cancer before the age of 40 by 60% and even beyond the age of 50 by 20%.

The authors present highly challenging aspects without the claim of solving the problems at issue. It is felt that the possible role of radiation doses, smoking, history, social status and of other factors in the production of a second carcinoma has to be established in the light of further studies.

The book will be of particular interest to oncologists and gynaecologists.

S. CSÖMÖR

Directory of On-going Research in Cancer Epidemiology. Editors C. S. MUIR, G. WAGNER. Lyon 1984. Price: £ 18.—

This volume, the 62nd number in this series is a joint publication by the WHO-sponsored International Agency of Cancer Research and the German Cancer Research Centre.

It falls into two distinct parts. The first provides concise information, reminiscent of the style of Excerpta Medica, on 1213 fields of research, condensed into 466 pages. It also

considers the keywords, the sites of the respective tumours, the start and possible completion of the studies.

From the statistical figures given in the introduction it emerges that in the framework of research in 1984 the new subjects have attained a proportion of 16.5%, this figure having shown a declining tendency since 1980. Between 1976 and 1980 new subjects of research were increasing in number. This part gives a global review representing the research teams of 61 countries.

In the second part which for the reader is no less important, information is grouped under the following aspects: name of authors in alphabetic order, keywords, sites of the tumours, chemical compounds under study, methods used, occupations under review. Subsequently the research teams of the various countries, the addresses of the cancer-registers based on various populations are listed for the facilitation of contacts between the research centres all over the world.

This well-arranged, carefully edited book meets its objective: it will be indispensable to all those seeking overall information on international research in cancer epidemiology. To-day there are certainly reliable informative services organized by the various centres engaged in cancer research, but this volume, as an aid on the bookshelf, is unique in its own kind.

S. CSÖMÖR

Laboratory Decontamination and Destruction of Carcinogens in Laboratory. Wastes: Some Hydrazines. IARC Scientific Publications No. 54. Lyon 1983. 87 pages. Price: £ 7.95

Some hydrazines have carcinogenic, some others, antineoplastic properties. The wastes, even though of small quantity, formed in the course of laboratory operations, must be decontaminated before disposal. In the pertinent literature we find but sparse information on the destruction of carcinogenic wastes, and even the published procedures leave some doubts about the reliability of testing. The procedures discussed in the book have been developed for small quantities, but are adaptable to massive wastes as well.

Hydrazine, monomethylhydrazine (MMH), 1,1-dimethylhydrazine (UDMH) and 1,2-dimethylhydrazine (SMDM) have been found carcinogenic to laboratory animals (dogs, mice). They are toxic to humans, but their carcinogenecity has not been confirmed. Procarbazine has been studied for carcinogenecity in mice.

After a review of the analytical procedures the degradation techniques are described. The explosion hazards obviously exclude the use of ashing methods. For the elimination of hydrazine wastes the following treatments are recommended.

1) potassium hydroxide solution of a nickel-aluminium alloy;
2) sulphuric K-permanganate;
3) K-iodate;
4) hypochlorite.

All procedures are described in detail. The efficiency of all these procedures attains 99%.

The annexes list the chemical and physical constants of the hydrazines, give the details of the essential biological and chemical procedures for the degradation of hydrazine and provide a bibliography.

The publication will be of use, in addition to research workers dealing with products of this kind, also to those being in charge of the disposal of such materials.

K. JOBST

Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes: Some N-Nitrosamides. IARC Scientific Publications No. 55. Lyon 1983. 65 pages. Price: £ 6.95

Biological and biochemical investigations concerned with the pathomechanism of carcinogenesis have made extensive use of N-nitrosamides. In the publication dealing with these compounds (IARC SP No. 43) various methods have been recommended for the decontamination of laboratory wastes, and these methods were expected to be practicable with N-nitrosamides as well. The experimental results have, however, shown that the procedures in question, though being suited for the degradation of N-nitrosamides, give rise at the same time to the formation of harmful mutagenic compounds. This makes direct application of the method unsafe, even in the case of related compounds.

Though it is uncertain whether and how far the N-nitrosamides under study occur in our environment, some of their representatives are used in diazoalkane synthesis [(N-nitroso-N-ethylurea (NNU), N-nitroso-n-methylurea (NMU)], in cancer therapy or in biological research (bischloroethylnitrosourea and 1(2-chloroethyl)3-cyclohexyl-1-nitrosourea).

MNU and ENU if applied in adequate doses produce tumours in practically all organs of laboratory animals. It is typical of these compounds to produce tumour in the brain and the nervous system and prevalently to affect young animals. The carcinogenicity of N-nitroso-N-methylurethane (MNUT) and of N-nitroso-N-ethylurethane (ENUT) has been also demonstrated. It is not known whether the N-nitrosamides are carcinogenic to humans as well.

Degradation of the N-nitrosamides with acid or alkaline solutions is unadvisable because of the production of toxic carcinogenic gases (diazomethane). The recommended procedures may be summed up as follows.

1) solutions of sulphanylic acid hydrochloride;
2) hydrochloric iron filings;
3) acid potassium permanganate;
4) denitrosilation with bromohydrogen.

Each procedure is described in detail. Destruction of N-nitrosamide contained in the samples attains 99.5%. The degradation products of some of the compounds examined are mutagenic to strains TI530 and TI535 of Salmonella typhimurium, depending on the decontamination method used.

The Appendix lists the nomenclature of the most common N-nitrosamides, their physical and cmical properties and gives a list of references relating to the biological and chemical decontamination methods.

The publication is essential to biologists engaged in experimental oncology, as well as to chemists and laboratory workers dealing with N-nitrosamides.

K. Jobst

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American Cancer Society
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The International Union Against Cancer, with funds provided by the American Cancer Society, will award fellowships for **research on cancer.**

The awards will be granted to experienced investigators who have demonstrated their ability for independent research and who wish to broaden their experience by a period of study **at a single** institution in another country.

Fellowships will be granted only to persons on the staff of universities, teaching hospitals, research laboratories or similar institutions.

Awards will be made to investigators who are devoting themselves either to the **experimental** or the **clinical** aspects of cancer research.

Fellowships will not be granted to persons who wish to perfect their training in methods of cancer detection or in therapeutic techniques, or who wish to visit briefly several institutions abroad.

The duration of fellowships will be one year but in special circumstances this period may be longer or shorter.

The stipend will be based on the current salary of the applicant and the salary of an investigator of comparable experience in the place where the applicant expects to study.

An allowance will be made towards the cost of travel of the fellow and of those dependants who will accompany him.

The deadline for receiving applications and supporting documents is 1 October. Successful applicants may begin their Fellowship at any time during the twelve months' period beginning 1 May.

Application forms and additional information may be obtained from:

International Union Against Cancer

3, rue du Conseil-Général
1205 Geneva
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The Yamagiwa-Yoshida Memorial International Cancer Study Grants

The Yamagiwa-Yoshida Memorial International Cancer Study Grants are funded by the Japan National Committee for the UICC which receives strong financial support from the Olympus Optical Company, Ltd, in Tokyo. These Study Grants are administered by the International Union Against Cancer.

They are designed to enable investigators of any nationality to gain experience in, or make comparative studies of, special techniques in both the biologicaland clinique aspects of cancer research.

The study grants will not be awarded for the purpose of visiting a number of institutes or of solely participating in congresses, conferences, and symposia.

They will be awarded for periods not exceeding 90 days.

Each grantee will receive a travel allowance towards the cost of a tourist/economy air fare, and a living allowance towards the cost of board and lodging. No allowance will be paid for dependents.

The closing dates for receipt of applications will be 30 June or 31 December of each year.

Successful applicants will be notified within 90 days of each closing date. Study Grants must be activated within 180 days of the date of notification.

Application forms and additional information may be obtained from:

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Synopsis of the three projects administered by the UICC

- American Cancer Society-Eleanor Roosevelt International Cancer Fellowships

- Yamagiwa-Yoshida Memorial International Cancer Study Grants

- International Cancer Research Technology Transfer Project (ICRETT)

Additional information and application forms may be obtained from :

International Union Against Cancer - 3, rue du Conseil-Général - 1205 Geneva (Switzerland)
Telephone (41-22) 20 18 11 - Telex 429 724

International Cancer Research Technology Transfer Programme

(ICRETT)

DATA BANK (ICRDB)

The International Union Against Cancer, with funds partly provided by the International Cancer Research Data Bank (ICRDB) of the National Cancer Institute of the United States of America, and partly by the International Union Against Cancer, will award "International Cancer Research Technology Transfer" grants for research on cancer.

The purpose of this programme is to promote direct and rapid person-to-person transfer of information about new or improved techniques or methods between investigators located in different countries who are working in areas of basic, clinical or behavioural research in order to further the progress of cancer research.

The available funds are designed to permit investigators of any nationality* to visit a research centre or centres abroad for a period not exceeding 28 days. The grant will be allocated towards travel and living expenses.

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INTERNATIONAL UNION AGAINST CANCER

rue du Conseil-Général 3, 1205 Geneva

Switzerland

* In accordance with US Federal Regulations, this Programme is not open to employees of US Government Agencies.

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INFORMATION FOR AUTHORS

Acta Medica Hungarica is published under the auspices of the Hungarian Academy of Sciences. Manuscripts and editorial correspondence should be sent to the editorial office: H-1450 Budapest 9, P.O. Box 67.

Original articles dealing with clinical and experimental medicine will be accepted with the understanding that they have not been and will not be published elsewhere and are subject to editorial revision.

Form of manuscripts

Two copies of the manuscript typewritten double-spaced with margins at least 4 cm wide should be submitted. Pages should be numbered consecutively. The first page should contain (1) the title of the paper (2) the initials and first name(s) of the author(s), (3) name of the institution where the work was done, (4) name and address of the author to whom correspondence and offprint requests should be addressed — this will appear as a footnote; (5) an abstract not exceeding 250 works which states the purposes of the study, the main findings and principal conclusions. Below the abstract provide 3 to 10 keywords that will assist indexers in cross-indexing the article.

The text of the paper should be divided into sections with the headings: Introduction, Materials (Patients) and Methods, Results, Discussion, References.

Unusual abbreviations should be identified in an alphabetical list typed after the abstract and keywords.

Drugs must be referred to by their WHO code designation (Recommended International Nonproprietary Names); use of proprietary names is unacceptable.

The international system of units (SI) should be used for all measurements.

References

These should be cited in the text as numbers in square brackets. The list of references should contain in alphabetical order of the first authors' names the following: authors' last names with initials; for journal articles the title of the paper (lower case), journal title abbreviated according to the style used in Index Medicus, volume number, inclusive page numbers, year of publication in parentheses; for books the title (upper and lower case), publisher, place and date of publication. Only manuscripts accepted for publication may be included in the reference list.

Examples:

1. Stagg, B. H., Temperly, J. M., Wyllie, J. H.: The fate of pentagastrin. Gut **12**, 825—829 (1971)
2. Falkner, F.: Prevention in Childhood of Health Problems and Adult Life. WHO, Geneva 1980.
3. Fishman, A. P.: Dynamics of pulmonary circulation. In: Hamilton, W. F., Dow, P. (eds): Handbook of Physiology. American Physiological Society, Washington 1963, pp. 65—79.

Tables

Each table should be typed on a separate sheet. They should be numbered consecutively with Roman numerals and have a brief specific title. The data presented in the table must be logically and clearly organized and should be self-explanatory. Omit internal horizontal and vertical rules. Cite each table in the text and indicate its approximate place on the margin.

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Figures should be submitted in duplicate. They must be numbered consecutively with arabic numerals. All figures should bear the name of the first author, the figure number and an arrow indicating the top. Cite each figure in the text and indicate its approximate place on the margin. If a figure has been published, acknowledge the original source and submit written permission from the copyright holder to reproduce the material. Figure captions should be submitted typed double-spaced on a separate sheet.

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