FORMERLY ACTA MEDICA ACADEMIAE SCIENTIARUM HUNGARICAE

Acta Medica Hungarica

VOLUME 40, NUMBER 1, 1983

Akadémiai Kiadó, Budapest
ACTA MED. HUNG. 40(1) 1–71 (1983) HU ISSN 0001–5989

ACTA MEDICA HUNGARICA

A QUARTERLY OF THE HUNGARIAN ACADEMY OF SCIENCES

Acta Medica publishes reviews and original papers on clinical and experimental medicine in English.

Acta Medica is published in yearly volumes of four issues by

AKADÉMIAI KIADÓ

Publishing House of the Hungarian Academy of Sciences
H-1054 Budapest, Alkotmány u. 21

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H-1083 Budapest, Szigony u 43 or H-1450 Budapest 9, P. O. Box 67

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Acta Medica is indexed in Current Contents

ACTA MEDICA

HUNGARICA

VOLUME 40

AKADÉMIAI KIADÓ, BUDAPEST

1983

NUMBER 1

NUMBERS 2—3

NUMBER 4

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Obstetrics

PROLACTIN SYNTHETIZING CAPACITY OF THE HUMAN DECIDUA DURING THE FIRST TRIMESTER OF PREGNANCY

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(Received May 17, 1982)

In vitro prolactin (PRL) synthesis by human decidua of early pregnancy (5–12 weeks gestation) was investigated. Tissues obtained during therapeutic abortions were incubated in oxygenated Gey's buffer supplemented with 20% fetal calf serum at 37 °C for 24 h. PRL production increased rapidly to 42.3 mU PRL/100 mg tissue wet weight until 8 weeks gestation and then slightly declined but these differences were not significant. There was no PRL production when chorion was incubated under similar conditions. A significant correlation was found when the initial PRL content in the decidua prior to incubation and the capacity of decidua to synthesize PRL were compared ($r = 0.76$, $P < 0.001$). These data provide further evidence that human decidua can produce PRL in vitro. The finding that the rate of the PRL synthesis increases progressively in early pregnancy is in correlation with the intensive decidualization of the endometrium.

Keywords: prolactin synthesis, decidua, early pregnancy

Introduction

Human decidua from term pregnancies has been reported to synthesize PRL in vitro [14]. This hormone was indistinguishable from pituitary PRL by immunologic, electrophoretic, chromatographic and receptor-assay techniques [5]. When chorion, amnion and fetal membranes were incubated, no significant amount of PRL accumulated in either the medium or the tissue [6, 13].

Since the endometrium undergoes decidualization during the late secretory phase even in the absence of conception, the decidualized endometrium was supposed to synthesize PRL. It has been proved that the ability of endometrium to produce PRL increases as the decidual reaction spreads throughout the endometrial stroma [10, 40].

As in early pregnancy (5–12 weeks gestation) there are data for the initial content of PRL in decidua [8], the purpose of the present study was to investigate the capacity of the human decidua to synthesize PRL in vitro during the first trimester of pregnancy.

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Materials and methods

Human decidua and chorion were obtained by curettage during therapeutic abortions from 5–12 weeks gestation, as calculated from the last menstrual period. A portion of each tissue was fixed in 4% formalin for histological examination, then embedded in paraffin wax, sectioned to 5 μm and stained with hematoxylin and eosin. Only those experiments were included in statistical analysis in which the decidual explants contained only decidual tissue and the chorionic explants contained only chorionic tissue. The specimens were washed in sterile, cold normal saline, minced and 500 mg aliquots were placed into Erlenmayer flasks containing 20 ml Gey's buffer [11] pH 7.4, supplemented with 20% fetal calf serum (Biological Research Centre, Szeged), penicillin 100 IU/ml, and streptomycin 100 μg/ml. Some flasks were frozen for determining later the preincubation PRL content in the decidua. The remaining flasks were bubbled with 95% oxygen and 5% carbon dioxide and incubated at 37 °C for 24 h. Flasks were sampled and regassed at 3, 6 and 20 h. Finally the incubated tissues were homogenized in the remaining buffer and the supernatants were assayed for PRL.

PRL concentration in the samples and tissue homogenates was determined by radioimmunoassay with double antibody separation. Human hypophysis PRL (National Institute for Biological Standards and Control, London), ^{125}I–PRL, rabbit antisera and anti-rabbit γ-globulin were received from the World Health Organization. The sensitivity of the assay was 65 mU/l. The intraassay coefficient of variation was 6.2; the interassay coefficient of variation was 12.9. Cross-reaction with HCG was 0.05% at B/B_0 of 50%. The PRL content of Gey's buffer containing 20% fetal calf serum was below 65 mU/l. Mean PRL production, i.e. the PRL concentration of the tissue and medium after incubation minus the PRL concentration of the tissue before incubation, was calculated for each group. Differences between these means were tested by Student's t test, with the significance defined as $P < 0.05$.

Results

PRL content in the incubation medium was progressively increasing during 24 h incubation at 37 °C. The accumulation of PRL in the incubated decidua specimens obtained from 6 weeks pregnancies is shown in Fig. 1.

Examining the PRL synthesis of 69 decidual specimens from 5–12 weeks gestation it was found that the capacity of the decidua to produce PRL increased till the 8th week of pregnancy reaching a maximum level of 42.3 mU PRL/100 mg tissue wet weight. PRL production did not change significantly

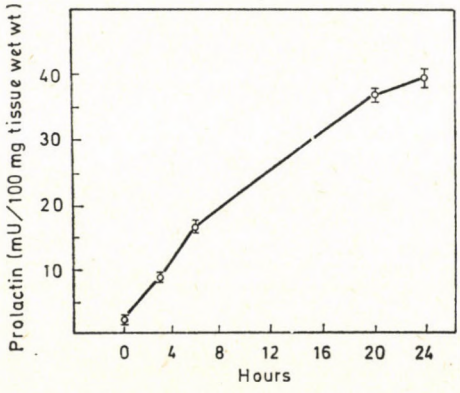

Fig. 1. Increase of PRL concentration in medium during 24 h incubation. Decidual tissue was obtained from patients of 6 weeks gestation. Values represent mean ±S.E.M.

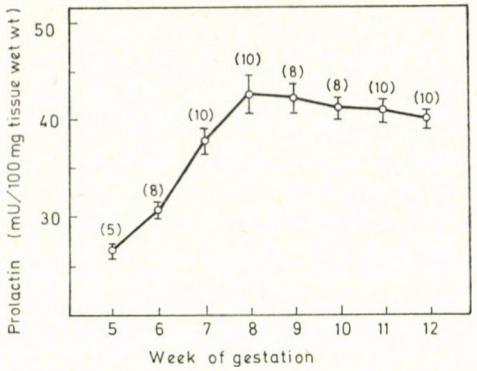

Fig. 2. In vitro PRL production by decidua throughout the first trimester of pregnancy. Values represent mean ±S.E.M. The number of decidual specimens in each week is shown in parentheses

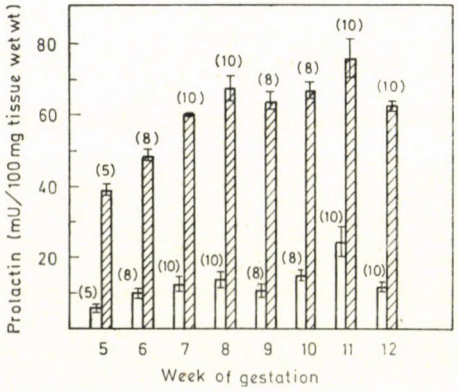

Fig. 3. Amount of PRL in incubation medium and tissue before (empty blocks) and after (striated blocks) 24 h incubation. The number of decidual specimens in each week is shown in parentheses. Each bar represents the mean PRL concentration ±S.E.M.

beyond that point. The amount of PRL produced by the decidua in vitro in the first trimester of gestation is presented in Fig. 2.

The initial PRL content in the decidua and the amount of hormone accumulated in the incubation medium plus tissue during 24 h incubation showed a similar pattern. Figure 3 shows the PRL concentrations in tissue homogenates before and after incubation at different stages of pregnancy.

Correlation between the preincubation content of PRL in decidual tissue and the capacity of the decidua to synthesize PRL was investigated. The linear regression is shown in Fig. 4; the correlation was 0.76 ($P < 0.001$).

When chorion was incubated, no significant amount of PRL accumulated in either the medium or the tissue. Both the initial content and in vitro production of PRL by chorion samples were considerably lower than that in decidua

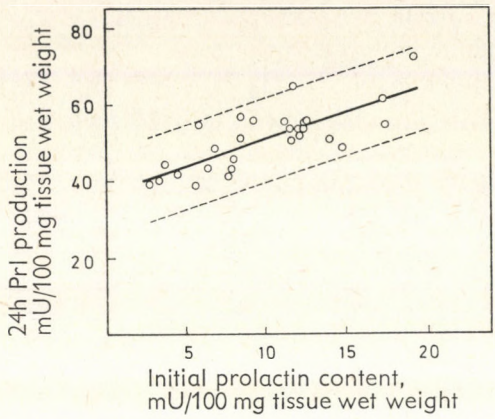

Fig. 4. Correlation of initial PRL content in decidual tissue and the ability of the decidua to synthesize PRL in vitro. Broken lines give the 95% confidence limit for the regression line ($y = 1.34x + 37.1$); $r = 0.76$, $P < 0.001$)

specimens. The chorion contained PRL in concentrations ranging from 0.26 to 0.66 mU/100 mg tissue wet weight prior to incubation and from 0.05 to 1.51 mU/100 mg tissue wet weight after incubation as determined in 10 experiments.

Discussion

Maternal serum PRL concentration rises steadily throughout gestation and reaches a ten times higher peak value than that in nonpregnant state. PRL level in amniotic fluid is 100 times higher in early pregnancy and 5 to 10 times higher at term than the corresponding maternal and fetal serum levels [7, 16]. The amniotic fluid PRL proved to be identical to pituitary PRL when judged by several physicochemical, biological and immunological criteria [1, 2, 12] but its origin and physiologic role was unknown until quite recently. An extrapituitary source of PRL was suggested by experiments in pregnant Rhesus monkeys demonstrating that maternal hypophysectomy or fetal death did not affect the concentration of PRL in amniotic fluid [17]. This hypothesis was supported by the observation that the PRL level was elevated in amniotic fluid but was suppressed in the maternal and fetal circulation during bromocriptine therapy [3].

Riddick et al. reported de novo PRL production by human decidua from term pregnancies and concluded that sufficient PRL was present in the decidua to account for that found in amniotic fluid [13]. PRL production in vitro could be blocked by protein synthesis inhibitors [6, 14].

A high correlation was found between the concentration of PRL in amniotic fluid and the capacity of the decidua to synthesize PRL during late

gestation. A similar correlation was seen when the amniotic fluid PRL levels and the initial hormone content of the decidua were compared [15].

Maslar and Riddick investigated the ability of the endometrium unassociated with pregnancy to produce PRL in vitro. They concluded that PRL was synthesized by the endometrium during the normal menstrual cycle and found a relation between the appearance and degree of synthesis and the decidualization of the stroma [10].

Endometrial tissue specimens from very early pregnancies contained and produced PRL in concentrations comparable to that released by the nonpregnant endometrium. Both the initial PRL content of the endometrium from 2 to 6 weeks gestation and the total hormone concentration measured in the tissue and medium after incubation increased rapidly as implantation progressed [9].

The immunoreactive PRL content of the decidua and the villi was determined in early pregnancy and the highest amount of PRL was found in decidual specimens of 8 weeks gestation. In one incubation experiment only the PRL release and not its production was observed during 2 h incubation [8]. De novo hormone synthesis was not examined.

In the present study, the ability of the decidua to synthesize PRL in vitro was investigated throughout the first trimester of pregnancy. The concentration of PRL produced by the decidua during 24 h incubation increased progressively from 5 to 8 weeks gestation; beyond that time no significant differences were noted. Chorion specimens did not produce PRL in vitro under identical conditions and the initial hormone content in these tissues was considerably lower than in the decidua. A significant correlation was found between the preincubation PRL concentration in the decidua and the ability of these tissues to synthesize PRL.

These data provide an additional evidence that the human decidua is capable of producing PRL in vitro and might be the source of amniotic fluid PRL. The rapid increase in hormone synthesis is related to the intensive decidual changes during the early stage of gestation. The possible role of this extrapituitary PRL remains open to further studies.

REFERENCES

1. Ben-David, M., Chrambach, A.: Preparation of bio- and immunoreactive human prolactin in milligram amounts from amniotic fluid in 60% yield. Endocrinology **101,** 250–261 (1977).
2. Ben-David, M., Rodbard, D., Bates, R. W., Bridson, W. E., Chrambach, A.: Human prolactin in plasma, amniotic fluid and pituitary: identity and characterization by criteria of electrophoresis and isoelectric focusing in polyacrylamide gel. J. clin. Endocr. Metab. **36,** 951–964 (1973).
3. Bigazzi, M., Ronga, R., Lancranjan, I., Ferraro, S., Branconi, F., Buzzoni, P., Martorana, G., Scarselli, G., Del Pozo, E.: A pregnancy in an acromegalic woman during bromo-

criptine treatment: effects on growth hormone and prolactin in the maternal, fetal and amniotic compartments. J. clin. Endocr. Metab. **48,** 9–12 (1979).
4. Golander, A., Hurley, T., Barrett, J., Handwerger, S.: Synthesis of prolactin by human decidua in vitro. J. Endocr. **82,** 263–267 (1979).
5. Golander, A., Hurley, T., Barrett, J., Hizi, A., Handwerger, S.: Prolactin synthesis by human choriondecidual tissue: a possible source of prolactin in the amniotic fluid. Science **202,** 311–313 (1978).
6. Healy, D. L., Kimpton, W. G., Muller, H. K., Burger, H. G.: The synthesis of immunoreactive prolactin by decidua-chorion. Brit. J. Obstet. Gynaecol. **86,** 307–313 (1979).
7. Hwang, P., Guyda, H., Friesen, H. G.: A radioimmunoassay for human prolactin. Proc. nat. Acad. (Wash.) **68,** 1902–1906 (1971).
8. Kubota, T., Kumasaka, T., Yaoi, Y., Suzuki, A., Saito, M.: Study on immunoreactive prolactin of decidua in early pregnancy. Acta endocr. (Kbh.) **96,** 258–264 (1981).
9. Maslar, I. A., Kaplan, B. M., Luciano, A. A., Riddick, D. H.: Prolactin production by the endometrium of early human pregnancy. J. clin. Endocr. Metab. **51,** 78–83 (1980).
10. Maslar, I. A., Riddick, D. H.: Prolactin production by human endometrium during the normal menstrual cycle. Amer. J. Obstet. Gynec. **135,** 751–754 (1979).
11. Porterfield, J. S.: A simple plaque-inhibition test for the study of Arthropod-borne viruses. Bull. WHO **22,** 373–380 (1960).
12. Rathnam, P., Cederqvist, L., Saxena, R. R.: Isolation of prolactin from human amniotic fluid. Biochim. biophys. Acta (Amst.) **492,** 186–193 (1977).
13. Riddick, D. H., Kusmik, W. F.: Decidua: a possible source of amniotic fluid prolactin. Amer. J. Obstet. Gynec. **127,** 187–190 (1977).
14. Riddick, D. H., Luciano, A. A., Kusmik, W. F., Maslar, I. A.: De novo synthesis of prolactin by human decidua. Life Sci. **23,** 1913–1921 (1978).
15. Rosenberg, S. M., Maslar, I. A., Riddick, D. H.: Decidual production of prolactin in late gestation: further evidence for a decidual source of amniotic fluid prolactin. Amer. J. Obstet. Gynec. **138,** 681–685 (1980).
16. Tyson, J. E., Hwang P., Guyda, H., Friesen, H. G.: Studies on prolactin secretion in human pregnancy. Amer. J. Obstet. Gynec. **113,** 14–20 (1972).
17. Walsh, S. W., Meyer, R. K., Wolf, R. C., Friesen, H. G.: Corpus luteum and fetoplacental functions in monkeys hypophysectomized during late pregnancy. Endocrinology **100,** 845–850 (1977).

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COMPARISON OF CARDIOTOCOGRAMS PREPARED BY ABDOMINAL ELECTROCARDIOGRAPHY AND BY ULTRASOUND CARDIOGRAPHY

(Recording and evaluation difficulties in the antepartum period)

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(Received April 8, 1982)

Cardiotocograms were prepared in 127 cases on 120 pregnant women, first by abdominal electrocardiography, and immediately afterwards by ultrasound cardiography. A study was made to ascertain which method yielded recordings of higher quality, and which factors influenced the quality of the cardiotocogram. It was found that the proportion of good-quality recordings was higher with ultrasound cardiography than with abdominal electrocardiography. The higher information content of the latter technique allows a more accurate assessment of the cardiotocograms. The recording quality is affected by numerous factors, the most important of which are adiposity and the duration of pregnancy. The use of abdominal electrocardiography is preferred in case of a thick abdominal wall, whereas the ultrasound technique is recommended between the 28th and 36th weeks of pregnancy.

Keywords: cardiotocogram, abdominal electrocardiography, ultrasound cardiography, antepartum period.

Introduction

A procedure widely used in antenatal diagnostics to assess the state of the foetus is the non-stress test (NST), based on the simultaneous examination of foetal heart function, uterine activity and foetal movements.

The foetal heart activity can be recorded by means of phonocardiography, ultrasound cardiography (uCG) and abdominal electrocardiography (aECG).

Owing to its disadvantages compared to the other two methods, phonocardiography is now rarely used in clinical practice [15]. uCG is a more con-

Abbreviations:

NST = non-stress test
uCG = ultrasound cardiography
aECG = abdominal electrocardiography
FHR = foetal heart rate
tr = transducer
EPH gestosis = toxemic manifestation of pregnancy
R = reactive
NR = non-reactive

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venient and simpler method, and it is therefore applied more widely than aECG. As regards the reliability of these procedures, few data are available on the simultaneous examination of the two methods on the same patient [1, 9, 15]. We have therefore made a comparative study of aECG and uCG recordings made consecutively on the same patient.

We sought answers to the following questions: Which method gives tracings of better quality? What are the factors influencing the quality of the cardiotocograms? Is there a difference in the evaluation of tracings of the same quality, depending on which method has been used?

Materials and methods

The NST was carried out on 127 occasions on 120 patients. For a given pregnant woman, the foetal heart rate (FHR) was recorded first by aECG, and immediately afterwards by uCG. The reason why the examinations had to be made in immediate succession is that it was not always possible to apply the ultrasound head, the transducer (tr) and the abdominal wall ECG electrodes to the best site simultaneously. A Kranzbüchler FM-5000 cardiotocograph was used, with a paper speed of 1 cm/min.

In aECG, ARBO disposable silver electrodes were used. The skin of the abdominal wall was first cleaned with alcohol, the upper layer of the epithelium was rubbed off with a Peerless device, Redux cream was rubbed into the skin to ensure good electrical conductance conditions, and the electrodes were located as shown in Fig. 1.

In uCG, the ultrasound tr was placed on the site corresponding to the maximum in the foetal heart sounds, and a conducting medium free of air bubbles was ensured with Aquasonic gel.

The positions of the electrodes and tr were varied until an appropriate signal was observed on the scope, or until the monitor sound detector revealed a rhythmic foetal heart function. The positions of the electrodes and the tr were not changed subsequently, even in the quality of the record deteriorated.

The mother-to-be indicated foetal movement by turning on a switch, and this was marked as a point in the upper part of the cardiotocogram.

Cardiotocograms were evaluated on the basis of criteria described earlier [2].

In the assessment of the quality of the recordings, we modified the original classification of Rüttgers and Kubli [14] by combining the groups of excellent and good quality. If the non-evaluable section did not exceed 15% of the length of the overall tracing, the quality was taken as good; for a ratio of 16–50%, the quality was considered satisfactory; and for values above 50%, the quality was regarded as non-evaluable.

The quality of the recording might be influenced by numerous factors, among which the following were examined: adiposity, duration of pregnancy, location of the placenta, quantity of the amniotic fluid, and number of foetal movements.

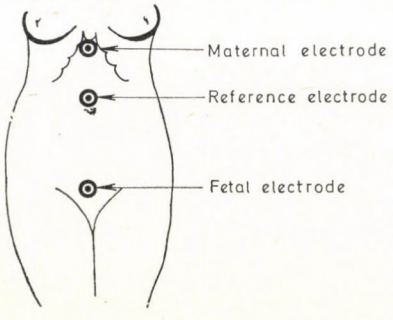

Fig. 1. Standard configuration of abdominal electrodes

Adiposity was calculated by the following formula: (100×body weight in kg)/(height in cm — 100). For values in the range 100±25% the body weight was considered normal, while higher values indicated obesity [10].

The duration of pregnancy was calculated on the basis of the last normal menstruation.

The location of the placenta was established by echography based on a Vidoson 635 B picture procedure. Three groups were distinguished: *(i)* placenta on anterior wall, *(ii)* placenta on posterior wall, *(iii)* measurement not performed, or placenta located elsewhere. In the case of placenta praevia, the patient was excluded from the comparative study.

The quantity of amniotic fluid was similarly determined by ultrasound echography. Two groups were differentiated: a normal quantity of amniotic fluid, or an elevated quantity of amniotic fluid (oligohydramnios did not occur in this study).

The number of foetal novements during the examination was counted by the patient; this number was considered enhanced if it was more than 30 in 30 min.

Various methods were used in the course of the statistical processing. In the groups of recordings of different qualities, the number of foetal movements was analysed using the Wilcoxon test.

The two-dimensional tables were examined with the χ^2 test; in the case of 2×2 tables, the Fisher test, too, was carried out since, mainly for low frequencies, this is a more suitable method.

Multivariant correlation — regression analysis — was performed to investigate how the quality of the uCG and aECG recordings, and their success rates, correlated with the other factors considered.

Results

The NST was carried out in 127 cases with aECG and then uCG immediately afterwards. Some of the examinations involved enhanced-risk pregnancies (EPH gestosis, postponed labour, and the suspicion of intrauterine growth retardation), while others were performed as screening examinations between the 28th and 36th weeks of pregnancy.

The recordings were classed into three groups using the criteria of Rüttgers and Kubli [14] as modified by us. These were: "good", "moderate" and "non-evaluable". Cardiotocograms of good quality could be obtained in 86% of the cases with uCG, and in 67% with aECG. The proportions for the recordings of moderate quality were 12% and 14%, respectively. A non-evaluable recording was obtained with the ultrasound technique in only one case, but on 24 occasions (19%) with aECG.

Obesity considerably influenced the quality of the recording for uCG. A similar correlation with aECG was not observed (Table I).

The duration of pregnancy did not affect the quality of the cardiotocogram in the case of uCG recordings, but when aECG was used the proportions of recordings of moderate or poor quality were significantly higher for the 28th–36th weeks than for the 37th–41st weeks (Table II).

Statistical methods did not reveal any influence of a placenta on the anterior wall on the quality of the cardiotocogram (Table III).

When analysing the role of the number of foetal movements, the Wilcoxon test did not demonstrate a significant difference between the numbers of foetal movements for the groups of recordings of moderate and of poor quality.

Table 1

Correlation between obesity and quality of cardiotocogram prepared with uCG or aECG

	uCG quality		aECG quality	
	good	moderate + poor	good	moderate + poor
Normal body weight*	88	8 (8+Ø)	64	32 (14+18)
Adipose*	23	8 (7+1)	21	10 (4+ 6)
Total	111	16 (15+1)	85	42 (18+24)

Fischer test: $P < 0.05$, $P > 0.05$
* Based on Broca index. For details, see text

Table II

Correlation between duration of pregnancy and quality of recording

Duration of pregnancy (weeks)	uCG quality		aECG quality	
	good	moderate + poor	good	moderate + poor
28–36	36	5 (5+Ø)	23	18 (4+14)
37–40	57	8 (8+Ø)	50	15 (8+ 7)
41–43	18	3 (2+1)	12	9 (6+ 3)
Total	111	16 (15+1)	85	42 (18+24)

Significance: $P > 0.05$, $P < 0.05$
χ^2 test (Yates correction): χ^2 0.061, χ^2 6.015
Degrees of freedom: 2

Table III

Correlation between location of placenta and quality of recording

Location of platenta	uCG quality		aECG quality	
	good	moderate + poor	good	moderate + poor
Anterior wall	48	8 (7+1)	38	18 (9+ 9)
Posterior wall	35	4 (4+Ø)	22	17 (7+10)
Other, or localization not performed	28	4 (4+Ø)	25	7 (2+ 5)
Total	111	16 (15+1)	85	42 (18+24)

Significance: $P > 0.05$, $P > 0.05$
χ^2 test: χ^2 0.339, χ^2 3.782
Degrees of freedom: 2

The influencing role of hydramnios was not analyzed by statistical methods owing to the small number of cases. In 5 of the 8 pregnancies complicated by hydramnios the quality of the aECG recording was moderate or poor, whereas uCG led to 5 recordings of good quality and 3 of moderate quality.

Multivariant regression analysis revealed a correlation between the quality of the recording and the factors influencing the quality of the cardiotocogram, with multiple correlation coefficients of 0.66 and 0.48 for aECG and uCG, respectively. In both analyses the significance level of the regression was high ($P < 0.001$).

To analyse the combined effects of the factors affecting quality, a table was prepared in which a study was made of whether the cases possessing 0, 1, 2 and 3 pathological parameters occurred in the same frequency for recordings of good quality and of moderate and poor quality. For both uCG ($P < 0.01$) and aECG ($P < 0.05$), the χ^2 test gave a significant result, i.e. the quality of the cardiotocogram depends on the number of the pathological parameters. The detailed data are presented in Table IV.

In the evaluation of the NST, three groups were formed: reactive (R), non-reactive (NR) and non-evaluable. Table V shows the distribution according to this classification.

It emerges from the tabulated data that the frequencies of the NR tests were almost the same with the two procedures. However, there were appreciable differences in the distribution of the reactive and non-evaluable tests; the explanation of this was sought by further analysis of the data. Within the group of cardiotocograms of the same quality, a study was made of the recording technique. It was found that for 79 recordings which were of good quality with both methods, the same classification was obtained in 61 cases (77%), whereas the test proved R with one method and NR with the other in 18 cases (23%). In 61% of these latter 18 cases, the uCG was considered to be R, while the aECG was NR. In the group of recording of moderate qual-

Table IV

Correlation between frequency of disturbing factors influencing the quality of the recording and the quality of the recording

Quality	Frequency of factors							
	aECG				uCG			
	0	1	2	3	0	1	2	3
Good	19	40	19	6	23	41	42	4
Moderate and poor	5	16	16	6	2	2	9	4

Significance: $P < 0.05$, $P < 0.01$
χ^2 test : $\chi^2 = 8.48$, $\chi^2 = 12.71$
Degrees of freedom: 3

Table V

Clinical evaluation of recordings

Evaluation	Procedure applied	
	uCG	aECG
Reactive (R)	102 (80%)	76 (60%)
Non-reactive (NR)	24 (19%)	27 (21%)
Non-evaluable	1 (1%)	24 (19%)
Total	127	127

ity, statistical analysis was not possible because of the low number (4) of cases. In 14 cases, aECG resulted in a cardiotocogram of moderate quality, while uCG on the same patients gave a tocogram of good quality; the reverse situation was observed in 6 cases. The clinical classifications were not subjected to comparison in these 20 cases, similarly as for the 24 recordings that could not be evaluated due to their poor quality.

Discussion

The clinical evaluation of cardiotocograms is fundamentally influenced by their quality. Repeated recording in case of non-evaluable tracings is a costly procedure. The additional costs caused by recording for a longer period because of moderate quality tracings must also be given consideration. We therefore first investigated how frequently cardiotocograms of good quality may be obtained by means of uCG and aECG.

Our experience is in agreement with the data of Solum [15] who obtained a result of good quality with uCG in 85% of the cases; our corresponding value was 87%. Using aECG, Solum produced recordings of good quality in 55.2% of the cases, compared with 67% in our study.

Of the factors adversely influencing the quality of the recording, obesity is of importance in the cases of uCG. Views differ concerning the role of a thick abdominal wall: Breuker et al, [4] and Rüttgers and Knbli [14] have proposed further investigations for clarification of the question, while Solum [15] maintains that the thickness of the abdominal wall does not influence the quality of the recordings. In our experience, a thick abdominal wall proved to be an impeding factor mainly for the ultrasound technique; with aECG there was no substantial difference.

It has been reported [14] that an anterior placenta impairs the quality of the recording, however this has not been confirmed by others. Our own

observations support the data of Solum [15]: we failed to find a significant correlation between the anterior location of the placenta and the poor quality of the recordings for either method, although the level of significance was almost reached for aECG.

The view is unanimous in the literature that aECG does nod yield a recording of suitable quality in the 28th to 34th week of pregnancy [3, 5, 8, 15]. Our observations indicate that it is more difficult to produce a recording of good quality with aECG in the 28th to 36th weeks of pregnancy. This was not the case when uCG was used. The quality of a cardiotocogram obtained with aECG may be improved by careful selection of the electrode sites. However, variation of the position of the electrode does not lead to a further essential improvement [5]. The quality of the recording is not affected by uterine contractions [5].

Hydramnios and the number of foetal movements are factors which were not studied in earlier publications. We assumed that these factors might have an unfavourable influence on the quality of the recording. The small number of cases does not permit us to draw any far-reaching conclusion as concerns hydramnios. It appears from our data that this disturbs the aECG technique more than the uCG recording. Clinical experience indicates that the quality of the recording frequently deteriorates at the time of intensive foetal movements. Using statistical methods we could not find a significant difference between the number of foetal movements measured in the group of recordings of good quality and in the group of moderate or poor quality; however, the average number of foetal movements was higher in the latter group.

Recording of the number of foetal movements may be rather unreliable: the attention of the patient may be affected by a recording of poor quality, while the number of foetal movements cannot be checked from the tocogram because of the poor quality [12].

Individual disturbing factors did not have a fundamental effect on the quality of the recordings, however several factors acting simultaneously did so. This was confirmed by multivariant correlation-regression analysis.

Indirect cardiotocography does not provide such an accurate picture as a recording obtained with a direct scalp electrode on the foetus [7]. Of the indirect methods, aECG has the highest information content and it better reflects the actual situation than uCG when using the direct method [15]. External monitoring "overestimates" the FHR variability [6, 13]. Paul and Keegan [11] reported that the uCG recording contained some accelerations which in fact did not exist, but Keirse et al. [7] could not confirm this.

Our own results were independent of the technique employed in 77% of the cases. Analysis of the 18 cases (a frequency of 23%) in which the results did not agree revealed that 61% of the cases classified as R by uCG proved NR on aECG. This fact supports the conclusions of Kierse et al. [7] referring

to the frequency of the accelerations and the erroneously recorded accelerations with the ultrasound technique. If different techniques are used, only recordings of the same quality may be compared.

Conclusions

1. The proportion of recordings of good quality is higher for uCG (86%) than for aECG (67%).

2. The quality of cardiotocograms obtained by aECG can be improved by careful selection of the site of the electrode; aECG is a more laborious method than uCG.

3. Because of its more accurate information content, aECG permits a differentiated assessment of the cardiotocograms, and it is therefore a useful method in antepartum cardiotocography. Its use is of benefit in all cases when accurate assessment of the oscillation is necessary, and thus when accurate assessment of the oscillation is necessary, and thus when an erroneously good oscillation (jitter) or oscillation constriction has been observed with uCG.

4. The quality of the recording is adversely affected by the thickness of the abdominal wall mainly with uCG, while the duration of pregnancy (28th–36th weeks) affects mainly the aECG; hence, the use of aECG is recommended in cases of adiposity, and the ultrasound technique in the early stages of pregnancy.

REFERENCES

1. Abelson, D., Balin H.: Analysis of the Doppler signals from the fetal heart. Am. J. Obstet. Gynecol. **112,** 796–801 (1972).
2. Bártfai Gy., Kovács L.: "Non stress teszt" új vizsgáló módszer az antenatalis diagnosztikában ('Non-stress test'. A new method in antenatal diagnosis). Orv. Hetil. **122,** 2470—2472 (1981).
3. Bolte, A.: Die pränatale fetale Elektrokardiographie. Gynäkologe **2,** 63–72 (1969).
4. Breuker, K. H., Khalili-Brunklaus, D., Bolte, A.: Das abdominale fetale EKG zur prä- und subpartualen Herz-Frequenzanalyse. Arch. Gynäkol. **221,** 211–235 (1976).
5. Carter, M. C., Gunn, P., Beard, R. W.: Fetal heart rate monitoring using the abdominal fetal electrocardiogram. Brit. J. Obstet. Gynaecol. **88,** 391–401 (1981).
6. Gross, T. L., Sokol, R. J., Rosen, M. G.: Clinical use of the intrapartum monitoring record. Clin. Obstet. Gynecol. **22,** 633–649 (1979).
7. Keirse, M. J. N. C., Trimbos, J. B., Mallens, T. E. J. M., van der Heemts, M.: Reliability of ultrasound as compared to direct fetal electrocardiography for antepartum cardiotocography. Brit. J. Obstet. Gynaecol. **88,** 391–394 (1981).
8. Klöck, F. K., Lamberti G., Schulte, H. J., Liedtke, B.: Die externe fetale Elektrokardiotokographie. Z. Geburtshilfe Perinatol. **176,** 81–83 (1972).
9. Klöck, F. K., Hartmer, L.: Die Kardiotokographie mit dem externen fetalen EKG im Vergleich zur Ultraschallkardiotokographie. Z. Geburtshilfe Perinatol. **181,** 178–188 (1977).
10. László, F. A.: In: Gláz, E. (ed.): Klinikai Endocrinológia (Clinical Endocrinology). Medicina, Budapest 1981, pp. 931–957.
11. Paul, R. H., Keegan, K. A.: Nonstress antepartum fetal monitoring. Clinics Obstet. Gynaecol. **6,** 351–358 (1979).

12. Pál A., Bártfai Gy., Gyöngyösi J.: A terhes által számolt és tocodynamométerrel észlelt magzatmozgás számainak egyezéséről (Correlation between the number of foetal movements counted by the patient and recorded by cardiotocography). Orv. Hetil. **123,** 1735–1737 (1982).
13. Pillay, S. K., Chik, L., Sokol, R. J., Zador, I. E.: Fetal monitoring: a guide to understanding the equipment. Clin. Obstet. Gynecol. **22,** 571–582 (1979).
14. Rüttgers, H., Kubli, F.: Kontinuierliche Registrierung von fetaler Herzfrequenz bei gleichzeitiger Wehenschreibung. II. Probleme der Instrumentierung. Gynäkologe **2,** 82–88 (1969).
15. Solum, T.: A comparison of the three methods for external fetal cardiography. Acta obstet. gynecol. scand. **59,** 123–126 (1980).

Acta Medica Hungarica, 40 (1), pp. 17—24 (1983)

Gastroenterology

EFFECT OF (+)-CYANIDANOL-3 (CATERGEN®) IN CHRONIC ACTIVE HEPATITIS

(Catergen® plus prednisolone versus prednisolone in a controlled study)

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(Received July 21, 1982)

Thirty-six patients with biopsy-proved chronic active hepatitis (CAH) were randomized. Of these 18 patients, 15 of them HBsAg and/or anti-HBc positive, received (+)-cyanidanol-3 (Catergen®, Zyma) in doses of 1.5 g daily for 6 months in addition to the previously started low dose (10–15 mg/day) prednisolone. The other 18 patients, 16 of them HBsAg and/or anti-HBc positive, continued on the former corticosteroid treatment exclusively. After 6 months an overall clinical-biochemical improvement was noted in 10 cyanidanol-treated and in 6 control patients, while the condition deteriorated in 4 drug-treated and 8 control subjects. Serum GPT activity decreased to less than twice the normal in 12/16 drug-treated and in 7/13 control subjects, gamma-GT fell to normal in 7/16 cyanidanol-treated and in 1/10 control patients, IgG fell to normal in 4/8 drug-treated and in 1/9 control patients who all had initially elevated values. The percentage of "active" E-rosette forming cells fell in both groups but the decrease was significant only in the control cases, i.e. cyanidanol inhibited the progressive fall in circulating T cells during the course of the disease. Lymphocyte proliferative response to phytohaemagglutinin stimulation, as measured by ^{3}H-thymidine incorporation, significantly increased in the cyanidanol group. Anti-HBs titres were markedly raised in 7/15 drug-treated and in 2/16 control patients.

Thus, cyanidanol had some slight beneficial effect on biochemical liver function tests and the immunological activity in CAH patients. Its use may be recommended as an adjuvant constituent of the complex therapy of the disease.

Keywords: (+)-cyanidanol-3, chronic active hepatitis

Introduction

The treatment of chronic active hepatitis, particularly of HBsAg-positive chronic active liver disease, presents many unresolved questions and contradictions despite the extensive use of corticosteroids. It has been shown that the flavonoid substance (+)-cyanidanol-3 (CY) (Fig. 1) is able to exert a variety of hepatoprotective actions in different types of liver damage [1] and may even accelerate the disappearance of HBsAg from the blood in

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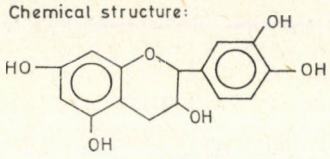
(+)-CYANIDANOL-3

Chemical structure:



(+)-3,4,5,7-tetrahydroxyflavan-3-ol
Empirical formula: $C_{15}H_{14}O_6$
Molecular weight: 290.28

Fig. 1

patients with acute and prolonged viral B hepatitis [2, 6]. Up to recent years only few data were reported on CY in chronic active hepatitis. In order to establish its value in that condition, a prospective controlled trial was performed in the years 1979 to 1981.

Patients and methods

Thirty-six patients with biopsy-proven chronic active hepatitis [3] previously treated with low doses prednisolone, 15 mg daily, were randomized into two groups: 18 patients, 15 of them HBsAg and/or anti-HBc positive, received CY in a dose of 1.5 g daily for 6 months, maintaining the previously started prednisolone therapy (CY group), while the other 18 patients, 16 of them HBsAg and/or anti-HBc positive, continued exclusively on the former prednisolone treatment (control group).

In every case the diagnosis was based on the findings of liver biopsy performed in 21 cases at the beginning of the study and at the end of the 6-month follow-up period.

Patients were checked at two-month intervals when beside a complete clinical examination several biochemical and immunological tests were performed.

Clinical symptoms and signs were anorexia, asthenia, abdominal pain, nausea, arthralgia, fatigue, hepatomegaly, splenomegaly, ascites, oedema and loss of body weight.

Laboratory studies consisted of white blood cell and platelet counts, and tests for serum bilirubin, aminotransferases (SGOT, SGPT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), pseudocholinesterase (PsCh), albumin, prothrombin activity, protein electrophoresis, quantitative IgG, IgA and IgM determination, antinuclear antibody (ANA), anti-smooth muscle antibody (SMA), hepatitis B surface antigen (HBsAg), anti-HBs and anti-HBc antibodies (measured by radioimmuno-assay AUSRIA, AUSAB, CORAB-Abbott), proportion of rapid (active) and late (total) E-rosette forming T cells as well as of EAC-rosette forming B cells, proliferative response of lymphocytes to phytohaemagglutinin (PHA) stimulation determined by ^{3}H-thymidine incorporation [9].

Histological changes were assessed by comparing the two liver biopsies in each subject who agreed to a repeated biopsy; 10 patients in the CY group and 11 in the control group were rebiopsied. The specimens were studied by the same pathologist who did not know of the nature of treatment. The histological features of the disease observed at the end of the trial were compared with the initial ones and classified as improved, unchanged or deteriorated.

All findings were evaluated after 6 months of treatment by comparing the initial and the final data in the two groups.

Statistical analysis was performed using Student's t test and chi-square analysis with Yate's correction.

Results

The overall course of the disease after 6 months was evaluated individually in each patient on the basis of the clinical signs and liver function tests. They revealed some differences between the two groups as regards improvement or deterioration, in favour of the CY group (Table I).

Table I

Overall clinical and biochemical condition (judged from physical signs and liver function tests)

	No. of patients			
	Total	Improved	Unchanged	Deteriorated
CY group	18	10	4	4
Control group	18	6	4	8
Total	36	16	8	12

Table II

Mean values ± S.E.M. of laboratory parameters before and after treatment

	CY (n=18)		Control (n=18)	
	before	after	before	after
Leukocytes, 10^9/l	5.0±0.4	5.6±0.4	5.3±0.4	5.1±0.3
GPT, IU/l	140±21	50±7**	139±24	46±7**
GGT	270±47	185±42	188±56	133±36
PsCh, U/l	255±25	283±30	283±32	248±30
Albumin, g/l	39±2	40±1	40±1	40±1
Prothrombin, %	72±4	80±4	83±4	78±3
γ-globulin, g/l	22±2	19±1	23±2	23±2
IgG, g/l	21±2	17±1*	20±2	20±2
IgA, g/l	3.1±0.3	3.3±0.7	3.0±0.3	3.3±0.2
IgM, g/l	2.7±0.3	3.2±0.5	1.8±0.2	1.9±0.2
Total T cells, %	64±4	58±9	59±4	57±4
Active T cells, %	47±6	44±5	41±5	28±3**
B cells, %	16±3	10±2	27±3	16±3**
PHA stimulation index	32±9	90±14**	33±10	54±17

** = $P < 0.05$, * = $P < 0.10$

Table II shows the mean values ±S.E.M. of some important laboratory variables obtained before and after treatment in the two groups. Table III presents the incidence of patients with pathological values at the beginning and at the end of the study.

No changes were noted in the serum bilirubin level in either group.

SGOT, LDH and ALP values showed no significant changes during the follow-up period, SGTP levels however, fell markedly in both groups. Although

Table III

Pathological values of biochemical and immunological indexes at the beginning and at the end of the study

	No. of patients with pathological values			
	(CY (n=18)		Control (n=18)	
	before	after	before	after
Leukopenia (<4.000)	7	1	5	4
GPT (>60 IU)	15	4	13	6
GGT (>50 IU)	16	9	10	9
PsCh (<220 U)	5/15	4/15	4/11	5/11
Albumin (<35 g/l)	7	4	5	4
Prothrombin (<75%)	11	7	8	7
γ-globulin (>25%)	11	7	11	13
IgG (>20.0 g/l)	8	4	9	10
ANA-positivity	9	8	9	8
SMA-positivity	7	6	8	6
Low active T cell ratio (<34%)	6/15	5/15	5/15	12/15
HBsAg-positivity	11	8	16	14
Anti-HBc positivity	13	10	12	11

Table IV

Histological changes after treatment

	No. of patients			
	Total examined	Improved	Unchanged	Deteriorated
CY group	10	1	5	4
Control group	11	0	7	4
Total	21	1	12	8

the post-treatment mean values did not differ, SGPT activity decreased from a pathological value to less than twice the normal in 12/16 CY and in 7/13 control patients. Similarly, GGT from an initially elevated level fell to normal in 7/16 CY and only in 1/10 control cases.

While the albumin level did not change, PsCh and prothrombin slightly decreased in the control and increased in the CY group, but these changes were not significant.

Serum IgG concentration fell from a pathological value to normal in 4/8 CY and 1/9 control cases. Furthermore, a marked (>4.0 g/l) decrease of the IgG level occurred in 7/18 CY and only in 2/18 control patients.

The percentage of "active" E-rosette forming T cells fell in both groups but the decrease was significant only in the control cases. Thus, CY seemed to inhibit the progressive fall in "active" T cells.

Lymphocyte proliferation in response to PHA-stimulation as measured by ^{3}H-thymidine incorporation significantly increased in the CY group.

HBV markers: serum HBsAg became negative in 3/11 CY and in 1/16 control cases, while the titre of anti-HBs antibody increased significantly in 7/15 CY but only in 2/16 control patients. Anti-HBc antibody titres became negative in 3/13 CY and in 1/12 control subjects. Anti-HBc titres increased in 2 CY and 4 control patients.

Histology revealed no significant difference between the two groups, as shown in Table IV.

During the trial we did not register any serious untoward effect. Mild gastrointestinal discomfort occurred in two CY patients but this did not necessitate the discontinuation of treatment.

After the end of the controlled study, two women with HBsAg-negative chronic active hepatitis who had not been involved in the trial, developed a Coombs-positive immune haemolysis. After prednisolone treatment and discontinuation of CY both became symptom-free.

Discussion

Our findings clearly reflect the contradictions concerning the therapeutic effect of CY in chronic hepatitis. The present results suggested a favourable action of the drug, although most of our data showed only trends and few statistically significant differences. Piazza et al. [10] reported on a marked difference between placebo and CY in chronic persistent hepatitis but not in chronic active hepatitis. Their persistent hepatitis patients who received the drug showed a significantly greater decrease in SGPT, GOT and gamma globulin levels than the patients on placebo, but this could not be observed in patients with active hepatitis.

Demeulenaere et al. [4] performed a controlled trial similar to our study; to chronic active hepatitis patients they administered prednisolone and either CY or placebo, according to a randomized table on a double-blind basis.

Unfortunately, we had no information about their work before starting the present study. While we estimate their sophisticated design as a good example of a therapeutic trial in chronic active liver disease, we agree with their view that it is ethically unacceptable to leave active hepatitis patients without any potentially effective treatment.

Demeulenaere et al. [4] found no difference between the two groups in the clinical and laboratory findings but noted a significant histological improvement as measured by the analysis of a number of parameters and by a quantitative evaluation of histological findings. On the other hand, we could observe some laboratory changes but no histological differences. This lack of correlation between the clinical-biochemical and the histological findings is not uncommon in chronic hepatitis.

In view of the reported mild therapeutic effect it should be emphasized that most of our patients involved in the study had been treated previously with low doses of prednisolone and so at the beginning of the study they were in a comparatively stable condition although not in complete remission.

An other problem is that of the dosage. While Piazza et al. [10] achieved good results in persistent hepatitis using CY in a daily dose of 3.0 g, as did also Di Nola [6] in prolonged viral B hepatitis. Demeulenaere et al. [4] and we ourselves administered daily doses of 1.5 g.

Most of our patients were HBsAg and/or anti-HBc positive — in other words their disease was related to HBV infection — while the data of the Belgian–French study [4] concerned mainly HBsAg-negative cases.

Our data failed to confirm the favourable effect of CY on the elimination of HBsAg. Although Kafkias et al. [7] and Di Nola [6] described such an action in acute or prolonged hepatitis B, Schomerus et al. [11] and others [13, 15] recently failed to support the pertaining observation of Blum et al. [2].

It may be relevant that we noted an increase in anti-HBs antibody titres more frequently in the CY than in the control group. This finding may reflect a favourable change in immune response against HBsAg due to the drug.

Some alterations in non-antigen-specific immunological reactivity also occurred, such as the more frequent decrease in serum IgG concentration, the inhibition of the progressive decrease in the proportion of circulating active T cells, and the significant increase in the proliferative response of lymphocytes to PHA stimulation in patients treated with CY.

All these immunological effects were in accordance with the in vitro results of Sipos et al. [12], Yamamoto et al. [16], Despont and Brögger [5], Vallotton and Frei [14], and similar to our findings obtained during levamisole therapy in HBsAg-positive acute and chronic active hepatitis [9].

Our experience agrees with those stating that CY is a non-toxic, well-tolerated drug. Yet, though not in this study, we observed two patients with CY induced immune haemolysis as did earlier Neftel et al. [8]. These cases will be reported in detail elsewhere.

In conclusion, CY has a slight beneficial effect on liver function tests and immunological activity in patients with chronic active hepatitis, although most of our findings mean rather trends than statistically significant differences. Definite proof concerning the real value of CY may be expected from further well-designed, properly controlled clinical trials and detailed immunological studies.

We do not believe that CY by itself could solve the question of treatment of HBsAg-positive chronic active hepatitis but we recommend its use as an adjuvant in the complex hepatoprotective, immunostimulant and antiviral chemotherapy of mild HBsAg-positive liver disease. In severe chronic active hepatitis it may also be valuable in combination with corticosteroid treatment.

Acknowledgements

We are indebted to Professor K. Jobst for performance of the biochemical liver function tests and to Dr. I. Hollós for anti-HBc determinations. Mrs Rózsa Kerekes and Miss Zsuzsa Maros carried out the immunoserological studies, Miss Zsuzsa Tatay performed the statistical analysis. Their expert assistance is gratefully acknowledged.

REFERENCES

1. Bertelli, A.: New Trends in the Therapy of Liver Diseases. Karger, Basel 1975, pp. 92–99.
2. Blum, A. L., Doelle, W., Kortum, K., Peter, P., Strohmeyer, G., Berthet, P., Goebell, H., Pelloni, S., Poulsen, H., Tygstrup, N.: Treatment of acute viral hepatitis with (+)-cyanidanol-3. Lancet **2**, 1153–1155 (1977).
3. De Groote, J., Desmet, V. J., Gedigk, P., Korb, G., Popper, H., Poulsen, H., Scheuer, P. J., Schmid, M., Thaler, H., Uehlinger, E., Wepler, W.: A classification of chronic hepatitis. Lancet **2**, 626–628 (1968).
4. Demeulenaere, F., Desmet, V. J., Dupont, E. Fiasse, R., Gisselbrecht, H., Heully, F., Jeanpierre, R., Lecomte, J., Lennes, G., Macinot, G., Migeotte, P., Pirotte, J., Rauber, G., Ruyters, L., Van Cauwenberge, H.: Study of (+)-cyanidanol-3 in chronic active hepatitis. Results of a controlled multicentric study. In: Conn, H. O. (ed.): International Workshop on (+)-cyanidanol-3 in Diseases of the Liver. Academic Press, London 1981, pp. 135–141.
5. Despont, J. P., Brögger, R. C.: Influence of (+)-cyanidanol-3 on human lymphocyte proliferation in vitro. In: Conn, H. O. (ed.): International Workshop on (+)-cyanidanol-3 in Diseases of the Liver. Academic Press, London 1981, pp. 111–112.
6. Di Nola, F.: (+)-cyanidanol-3 in acute viral hepatitis. Lancet **2**, 1379–1380 (1980).
7. Kafkias, B., Papachristou, A., Hatzidimou-Moula, M., Zachos, J., Kambourakis, G., Faltsis, S.: The treatment of hepatitis B and other liver diseases with (+)-cyanidanol-3. In: Conn, H. O. (ed.): International Workshop on (+)-cyanidanol-3 in Diseases of the Liver. Academic Press, London 1981, pp. 93–96.
8. Neftel, K. A., Diem, P., Gerber, H., de Weck, A. L., Stucki, P.: Durch (+)-cyanidanol-3 (Catergen) induzierte immunhämolytische Anämie. Schweiz. med. Wschr. **110**, 380–382 (1980).
9. Pár, A., Barna, K., Bajtai, G., Hollós, I., Ambrus, M., Kovács, M., Jávor, T.: Levamisole treatment of acute viral hepatitis and HBsAg-positive chronic active hepatitis. Acta med. Acad. Sci. hung. **37**, 269–281 (1980).

10. Piazza, M., De Mercato, R., Guadagnino, V., Picciotto, L., Abresia, N., Chirianni, A., Golden, G.: Effect of (+)-cyanidanol-3 on chronic persistent and chronic active hepatitis. In: Conn, H. O. (ed.): International Workshop on (+)-cyanidanol-3 in Diseases of the Liver. Academic Press, London 1981, pp. 124–129.
11. Schomerus, H., Wiedmann, K. H., Dölle, W., Peerenboom, H., Strohmeyer, G., Balzer, K., Goebell, H., Dürr, G., Bode, C., Blum, A. L., Frösner, G., Gerlich, W., Berg, P. A., Dietz, K.: (+)-cyanidanol-3 in acute viral hepatitis. In: Conn, H. O. (ed.): International Workshop on (+)-cyanidanol-3 in Diseases of the Liver. Academic Press, London 1981-pp. 85–87.
12. Sipos, J., Gábor, V., Tóth, K., Bartók, K., Ribiczey, P.: In vitro effect of (+)-cyanidanol-3 on rosette formation. In: Conn, H. O. (ed.): International Workshop on (+)-cyanidanol-3 in Diseases of the Liver. Academic Press, London 1981, pp. 113–115.
13. Theodoropoulos, G., Dinos, A., Dimitriou, P., Archimandritis, A.: Effect of (+)-cyanidanol-3 in acute viral hepatitis. In: Conn, H. O. (ed.): International Workshop on (+)3-cyanidanol-3 in Diseases of the Liver. Academic Press, London 1981, pp. 89–91.
14. Vallotton, J. J., Frei, P. C.: Influence of (+)-cyanidanol-3 on the leukocyte migration test carried out in the presence of PPD and HBs Ag. In: Conn, H. O. (ed.): International Workshop on (+)-cyanidanol-3 in Diseases of the Liver., p. 109. Academic Press, London 1981.
15. Wellmann, W., Vido, I., Schmidt, F. W., Müller, R., Ranft, U., Wildhirt, E., Holzer, E., Wallnöfer, H., Korb, G.: Influence of (+)-cyanidanol-3 on the course of acute viral hepatitis. In: Conn, H. O. (ed.): International Workshop on (+)-cyanidanol-3 in Diseases of the Liver. Academic Press, London 1981, pp. 83–84.
16. Yamamoto, S., Suzuki, H., Oda, T.: Immunological properties of (+)-cyanidanol-3. In: Conn, H. O. (ed.): International Workshop on (+)-cyanidanol-3 in Diseases of the Liver., pp. 143–144 Academic Press, London 1981.

Acta Medica Hungarica, 40 (1), pp. 25–31 (1983)

Cardiology

AUTONOMIC SINUS NODE DYSFUNCTION DOCUMENTED BY HOLTER MONITORING STUDIES IN 21 PATIENTS

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(Received October 28, 1981)

Holter recordings were carried out in 21 patients with presupposed sinus node dysfunction (SND). All the patients were evaluated also by complex electrophysiological examinations (overdrive and premature atrial stimulation) before and after pharmacologic autonomic blockade (AB) with propranolol 0.2 mg/kg and atropine 0.04 mg/kg body weight intravenously. Groups of normal and abnormal intrinsic heart rate (IHR) were compared. In patients with normal IHR (n=13) the prolonged recovery time (5/13), postpacing SA-block (1/13) and chaotic postextrasystolic patterns (4/13) ceased after AB. Intrinsic recovery time became normal with a gradual return to the stable sinus cycle length, and we obtained normal biphasic postextrasystolic intrinsic return responses. In all cases of pathological IHR (n=8), abnormal intrinsic rhythmicity was verified by electrophysiological means. Holter monitoring revealed significant differences ($P < 0.001$) between the minimal HR during sleeping and also in the HR averaged for 24 h between the two groups. In patients with abnormal IHR, the 24 h rhythm recording revealed positive ECG data for SND. In patients with normal intrinsic electrophysiological properties, Holter monitoring revealed severe sinus bradycardia (1 case), SA-arrest (1 case), SA-block (1 case), tachy-brady syndrome (1 case), and atrial fibrillation with AV-block (1 case). For appreciating the role and significance of the autonomic neuro-vegetative tone in SND, continuous rhythm monitoring is necessary.

Keywords: autonomic sinus node dysfunction, autonomic blockade, continuous ECG monitoring

Abbreviations:

SND:	sinus node dysfunction
AB:	autonomic blockade
IHR:	intrinsic heart rate
PPC_1:	first postpacing cycle (recovery time)
$CPPC_1$:	corrected PPC_1
PPC 2–10:	postpacing cycles 2–10 (secondary cycles)
SACT:	sinoatrial conduction time
SCL:	sinus cycle length
bpm:	beats/min

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Introduction

This report presents data concerning sinus node activity in patients with suspected sick sinus syndrome. The electrophysiological and continuous ECG monitoring data were compared to investigate the real usefulness of these criteria in order to gain a better recognition of concealed autonomic SND.

Patients and methods

Criteria for inclusion in this study were (1) history of sick sinus syndrome; (2) ECG manifestations referring to SND; (3) no history of heart failure, glaucoma, severe pulmonary disease. Twenty-one patients aged between 21 and 72 years (11 males and 10 females) were studied. Cardioactive drugs were discontinued for an interval exceeding three drug half-lives. Overdrive pacing was carried out for 30 s at 90, 120, 150 and 180 bpm. The duration of each of the ten postpacing cycles (PPC 1–10) was measured [16]. Sinoatrial conduction time (SACT) was estimated by Strauss methodology [13]; normal values for the sum total of SACT was $\leq$ 200 ms [1]. After control measurements and determination of the intrinsic heart rate (IHR), overdrive and premature stimulation were repeated in less than 25 min of pharmacological autonomic blockade. Autonomic blockade (AB) was determined as proposed by Jose [9] and modified by Jordan et al. [8]. Holter recording was carried out partly on hospitalized patients and partly under ambulatory conditions. The tapes were analysed with Oxford Medilog ECG system. A Hewlett Packard ECG arrhythmia evaluating instrument connected to the equipment indicated hourly minimal and maximal heart rate (HR), and average sinus cycle length (SCL/ms). Groups of normal and pathological IHR patients were compared. Results are expressed as mean $\pm$SD. Statistical analysis was performed by Student's *t* test.

Results

In group I, the IHR was normal, it fell within the 95% confidence limit of the predicted heart rate. All the 13 patients had symptoms. The average age was 43$\pm$6 years. In 6 patients the routine ECG referred to SND. Group II with abnormal IHR included 8 patients who had clinical and ECG marks indicating SND. Their mean age was 67$\pm$3.5 years.

Atrial postpacing responses. In group I the $CPPC_1$ was 320$\pm$270 ms; it was pathological ($>$325 ms) in five patients. Secondary abnormalities (SA-block) were observed in one patient. After AB the stability of SCL was striking, the maximal $CPPC_1$ was 190$\pm$56 ms, while the return to the basic intrinsic SCL was gradual, following the pattern of an exponential curve. In group II the maximal $CPPC_1$ (520$\pm$310 ms) was normal in 3 patients. In PPC 2–10 severe bradycardia appeared in 1 case. After AB, stabilization of the intrinsic SCL was observed in 2 of 8 patients, mild sinus arrhythmias occurred in 6 of 8 patients, while the $CPPC_1$ was significantly prolonged, 2248$\pm$2850 ms, markedly longer than 240 ms, considered as upper normal limit after the drug test [1]. The postpacing cycles failed to show a gradual exponential return to the intrinsic SCL.

SACT was estimated in 7 patients in group I (mean 206, range 100 to 242 ms) and chaotic pattern was observed in 6 patients because the duration

of the return cycle was inconsistent. After AB we could estimate the intrinsic SACT in all 13 cases (mean: 160 ms, range 86 to 238 ms). In group II, prolonged SACT was observed in 4 patients and chaotic in 1 patient. After AB an abnormal postextrasystolic pattern was found in 7 patients (4 chaotic and 3 prolonged intrinsic SACT).

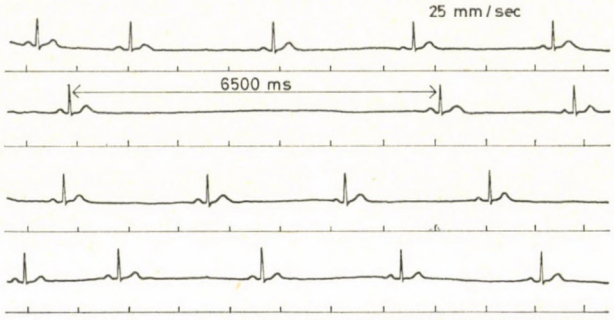

Fig. 1. Continuous Holter monitoring (25 mm/s). Autonomic bradycardia 24 bpm heart rate and SA-arrest (6500 ms) in a 26-year-old male with history of frequent dizziness. The intrinsic electrophysiological properties of the sinus node were normal

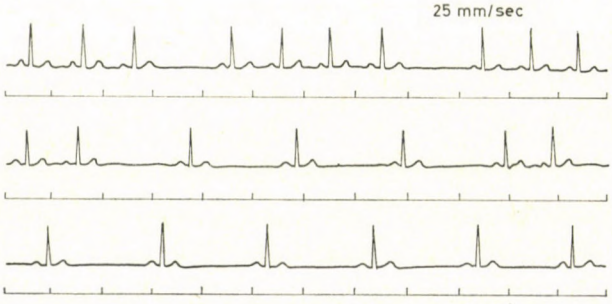

Fig. 2. Continuous ECG recording (25 mm/s) showed SA block in a patient with weakness and frequent dizzy episodes, normal IHR and normal electrophysiological postpacing data

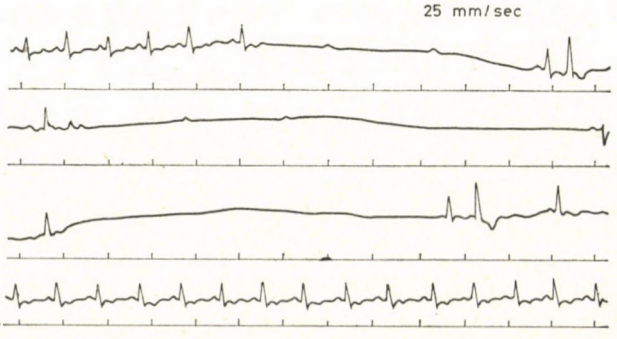

Fig. 3. Continuous rhythm recording (25 mm/s) carried out during a typical syncopal attack in a patient with normal intrinsic rhythmicity. Sinus rhythm has stopped, a high degree AV-block with complete SA-block appeared resulting in cardiac arrest

Holter recording. Total recording time for one patient was 1436±102 min in 24 h. In group I minimal waking HR was 53±9 bpm, the maximal 100± 52 bpm, while minimal sleeping HR was 46±22 and the maximal 102±38 bpm. In group II, the value of minimal HR was 40±10 bpm, and the maximal 102± 26 bpm during the waking period, while in the sleeping period the minimal HR was 34±6 bpm and the maximal HR was 86±32 bpm. There was no significant difference in the HR of the two groups concerning the maximal waking and sleeping period, but in the waking period concerning the lowest HR the P was < 0.05. A more significant difference was observed between the minimal HR during sleep ($P < 0.001$) and the mean SCL for 24 h ($P < 0.001$).

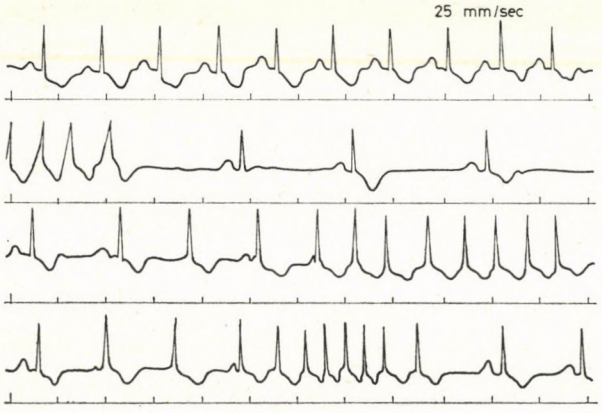

Fig. 4. Holter monitoring during tachy-bradycardia syndrome occurring both in daytime and at night

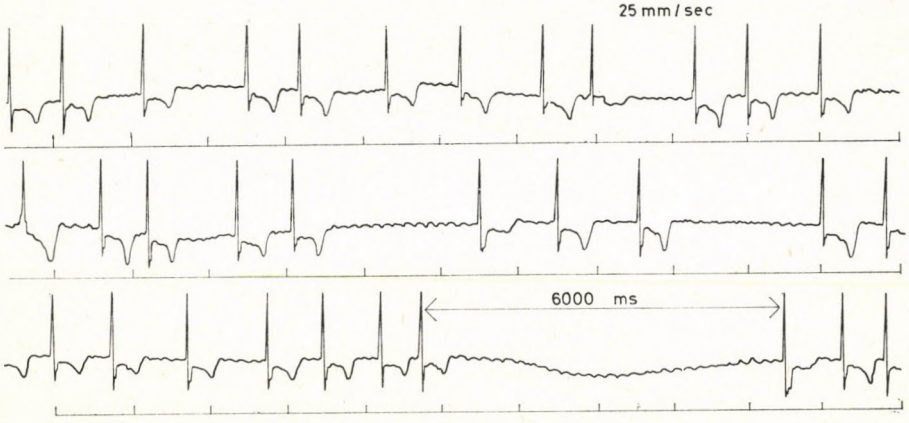

Fig. 5. Atrial fibrillation on continuous strip, with slow ventricular responses and frequent appearance of AV-block (the longest pause was 6000 ms) in a 42-year-old patient without any organic heart disease, with normal intrinsic electrophysiological properties of the sinus node and conduction structures of the heart

In group I, Holter recording revealed severe bradycardia (1 case, Fig. 1), SA-block (1 case, Fig. 2), SA-arrest (1 case, Fig. 3), tachy-brady syndrome (1 case, Fig. 4), atrial fibrillation and II° AV-block (1 case, Fig. 5). In group II, intermittent severe bradycardia was found in all examined patients, tachy-brady arrhythmia was alternating in four patients. Pauses ranging from 2760 ms to 8000 ms were observed in 4 patients.

Discussion

Prolonged rhythm monitoring may give more help in the diagnosis of sick sinus syndrome than the standard electrocardiogram [4, 6, 10, 12], but multiple diagnostic procedures are often necessary to reveal this heterogeneous and complex dysrhythmic entity [6, 12, 14].

Combined autonomic blockade and electrophysiological studies have increased the understanding of the normal and pathological sinus node activity [1, 2, 11, 14–22]. By autonomic blockade, however, we eliminate from the examination the functional neuro-vegetative factor which is of importance in the genesis of dysrhythmias and symptoms.

The present study suggested that in patients with abnormal IHR the intrinsic rhythmicity was always affected. In these cases the electrophysiological parameters were found to be abnormal in every patient, as described previously [8, 11, 16]. In addition the results of Holter monitoring showed positive ECG signs for SND in all cases.

If the IHR is normal the diagnosis of SND is more difficult. In these cases we eliminated by electrophysiological studies the intrinsic injuries of the pacemaker cells (normal IHR, stable SCL, normal intrinsic recovery time, exponential return to basic stable intrinsic sinus cycles, biphasic intrinsic postextrasystolic return patterns), but we found ECG's of severe sinoatrial dysrhythmia (Figs 1–5) concomitant with symptoms in the waking period patients. Holter monitoring carried out in healthy individuals proved that bradycardia without symptoms (lowest HR: 37 ± 4 bpm) and sinus pauses (the longest P–P interval was 2760 ms) may occur in different ages, predominantly during the vagotonic sleeping period [3, 5, 7, 23]. Normal intrinsic electrophysiological properties and positive Holter monitoring data suggested that the severe SND was caused by an imbalance in neuro-vegetative tone. In these cases of autonomic sinus node dysfunction, pharmacological denervation eliminated the most important pathogenetic factor from our electrophysiological examinations. If for the causation of the SND a pure vegetative factor is responsible, the properties of the pacemaker cells are physiological. Therefore continuous rhythm monitoring is of utmost importance in the diagnosis of autonomic SND.

Acknowledgments

We are indebted for technical assistance to B. Tildy and Mrs. Lomascolo, for secretarial assistance to Miss Forgách.

REFERENCES

1. Alboni, P., Malcarne, C., Pedroni, P., Masoni, A., Narula, O. S.: Electrophysiology of normal sinus node with and without autonomic blockade. Circulation **65,** 1236–1242 (1982).
2. Barnay, C., Hanvic, G., Szatmáry, L., Pisapia, A., Coste, A., Medvedowsky, J. L.: Study of sinus node function by programmed atrial stimulation after pharmacological autonomic blockade. International Symposium on Cardiac Arrhythmias, Abstr. p. 14, Marseille, 1982.
3. Brodsky, M., Wu, D., Dénes, P., Kanakis, C., Rosen, K. M.: Arrhythmias documented by 24 hours continuous electrocardiography monitoring in 50 male medical students without apparent heart disease. Amer. J. Cardiol. **39,** 390–395 (1977).
4. Crook, B. R., Cashaman, P. M., Scott, R. D.: Tape monitoring of electrocardiogram in ambulant patients with sinoatrial disease. Brit. Heart J. **35,** 1009–1013 (1973).
5. Djian, P., Egre, A., Bory, M., Savin, B., Mostefa, S., Serradimigni, A.: L'enregistrement électrocardiographique chez les sujets normaux. Arch. Mal. Cœur **72,** 655–661 (1979).
6. Ferrer, M. I.: The Sick Sinus Syndrome. Futura Publishing Co. Inc., New York 1974.
7. Fleg, J. L., Kennedy, H. L.: Cardiac arrhythmias in a healthy elderly population. Chest **81,** 302–307 (1982).
8. Jordan, J., Yamaguchi, I., Mandel, W. J.: Studies on the mechanism of sinus node dysfunction in the sick sinus syndrome. Circulation **57,** 217–223 (1978).
9. Jose, A. D.: The effect of combined sympathetic and parasympathetic blockade on the heart rate and cardiac function in man. Amer. J. Cardiol. **18,** 476–482 (1966).
10. Lipski, J., Cohen, L., Espinoza, J.: Value of Holter monitoring in assessing cardiac arrhythmias in symptomatic patients. Amer. J. Cardiol. **37,** 102–107 (1976).
11. Medvedowsky, J. L., Szatmáry, L., Barnay, C., Coste, A., Pisapia, A.: Étude de la dépression sinusale primaire et secondaire après stimulation auriculaire. Intérêt d'une épreuve pharmacologique de blocage du système nerveux végétatif. Ann. Cardiol. Angéiol. **30,** 395–402 (1981).
12. Reiffel, J., Bigger, J., Cramer, M., Reid, D.: Ability of Holter electrocardiographic recording and atrial stimulation to detect sinus node dysfunction in symptomatic and asymptomatic patients with sinus bradycardia. Amer. J. Cardiol. **40,** 189–194 (1977).
13. Strauss, H. C., Saroff, A. L., Bigger, J. T., Giardina, E. G.: Premature atrial stimulation as a key to the understanding of SA conduction in man. Circulation **47,** 86–93 (1973).
14. Szatmáry, L.: Effet électrophysiologique de la stimulation auriculaire rapide et du blocage combiné par atropine et propranolol du système nerveux autonome chez les patients atteints d'un dysfonctionnement sinusal. Mémoire de cardiologie, Ed. Méd. et Universitaires Marseille, 1979.
15. Szatmáry, L.: Electrophysiological effect of overdrive suppression and combined autonomic blockade with atropine and propranolol in patients with SND. 8th European Congress of Cardiology, Abstr., p. 32, Paris 1980.
16. Szatmáry, L., Veress, G., Borbola, J., Elkafarna, A., Zorándy, A., Czapkó, M.: The secondary poststimulative phase after rapid atrial pacing: its importance in the diagnosis of sinus node dysfunction. Acta med. Acad. Sci. hung. **38,** 97–107 (1981).
17. Szatmáry, L., Medvedowsky, J. L., Barnay, C., Medvedowsky, A., Pisapia, A., Delaage, M., Veress, G., Pinot, J. J., Jouve, A., Torresani, J.: Electrophysiological classification of normal and pathological sinus node function. Acta med. Acad. Sci. hung. **39,** 32–43 (1982).
18. Szatmáry, L., Medvedowsky, J. L., Barnay, C., Pisapia, A., Vincey, J. C., Coste, A., Auberge, J., Medvedowsky, A.: Part of the autonomous nervous system in the development of chaotic post-extrasystolic picture. Electrophysiological and pharmacological studies of 49 cases. Acta med. Acad. Sci. hung. **39,** 27–31 (1982).
19. Szatmáry, L., Barnay, C., Medvedowsky, J. L., Pisapia, A., Vincey, J., Coste, A., Medvedowsky, A.: Synchronized and desynchronized sinus node activity. Acta Cardiologica **37,** 345 (1982).

20. Szatmáry, L., Barnay, C., Hanvic, G., Saadjian, A., Torresani, I., Ebagosti, A., Veress G: Comportamento del ciclo di ritorno dopo blocco neurovegetativo in sogetti con disfunzione sinusale. G. Italiano Cardiol. Accepted for publication.
21. Vallin, H. O.: Autonomous influence on sinus node and AV node function in the elderly without significant heart disease: assessment with electrophysiological and autonomic tests. Cardiovasc.-Res. **14**, 206–215 (1980).
22. Vallin, H. O., Edhag, O., Sowtone, E.: Diagnostic capacity of sinus node recovery time after inhibition of autonomous neural tone. Eur. J. Cardiol. **12**, 81–93 (1980).
23. Viitasalo, M. T., Kala, R., Eisalo, A.: Ambulatory electrocardiographic recording in endurance athletes. Brit. Heart J. **47**, 213–220 (1982).

Acta Medica Hungarica, 40 (1), pp. 33—40 (1983)

Neurophysiology

EVENT-RELATED NON-SPECIFIC RESPONSES (K-COMPLEXES) DURING SLEEP

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(Received May 7, 1981)

EEG responses to sensory stimuli were obtained by averaging. In averages representing the reactivity of the approx. 5 min sleep period, the fluctuation of the amplitude of the obligatory part was evaluated. The deepening of sleep was reliably indicated by the increase in amplitude while the lightening of sleep by a decrease in amplitude within stage 2. In longer sleep periods beside the above tendency a micro-fluctuation occurred as evidence of the fine fluctuations of the sleep level within the phases. The synchronization response could be followed by this method even in the deep sleep stages and the above behaviour of the amplitudes of averages remained unchanged. This shows that the synchronization response to areousal stimuli is a uniform phenomenon during the whole slow-wave sleep. Our results support the assumption that the spontaneous or evoked synchronization phenomena in sleep are the products of the dynamic balance in the sleep-arousal system. An increase of amplitude may indicate an increase in the tonus of the sleep system while its decrease an opposite process. The present observations, similarly to our earlier results, may be adapted well to the sleep model based on the phasic activation and reciprocal induction theory.

Keywords: sleep, K-complexes, EEG

Introduction

It has been shown earlier that the characteristics of the electric parameters in sleep stages depend on the position of the sleep stages within the sleep cycle [3]. During these examinations it was attempted to interpret the dynamics of certain phasic events in the framework of the reciprocal induction model of the sleep process [8]. According to this concept the synchronization reactions were interpreted as the rebound of the sleep system, the electrodermographic discharges as phasic activation of the reticular arousal system [5] as well as the microarousals as a breaking through of awakening during the sleep process [4]. Another important phasic event, the sleep spindles, have not been investigated by us but the majority of authors attribute to them a sleep defending function [6, 13].

In the course of slow-wave sleep (SWS) the synchronization tendency is obvious; proceeding from superficial sleep towards deep sleep the amplitude

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is increasing while the frequency is decreasing. This is realized by the increasing appearance of phasic activity such as vertex sharp waves, K-complexes, slow-wave groups. A challenging feature of these phenomena is their ambiguous character: while they are standard elements of spontaneous sleep, the same phenomena may be evoked by sensory stimuli. There is some evidence of the connection of so-called spontaneous K-complexes and visceral events [9, 10].

We assumed that under the influence of arousal stimuli as well as in the spontaneous sleep process the EEG responses are caused by clashes between the sleep promoting and the arousal system. A paradoxical fact fits in well with this assumption namely that the so-called arousal response phenomena elicited in different stages of sleep are usually synchronization responses, hence they seem to be sleep responses. According to our interpretation, under prolonged dominance of sleep system only the "rebound" of sleep system is manifest from the "clash" of the two systems. (In contrast, in the waking state, i.e. under opposite conditions of dominance, the arousal stimuli entail desynchronization response phenomena.) The interaction of sleep and arousal system is working in sleep according to the reciprocal induction regulating principle. Therefore, with increasing activity of one "half system", the activity of the other "half system" of opposite function is also increasing and vice versa.

In the present work two problems have been studied:

1. Whether in certain sleep phases the synchronization responses to arousal stimuli are different or not. If they are, are the observed differences connected with the progress of sleep and with the changing balance in the sleep-arousal system? Would, for example, the differences between responses within the same sleep stage show opposite tendencies depending on the place of the given sleep stage in the sleep cycle, since in the descending part of the cycle the given phase is followed by a deeper, whereas in the ascending part by a more superficial phase of sleep?

2. It has also been studied whether the synchronization response may be followed even in the deep phases of slow-wave sleep (stages 3, 4) because it is so intermingled with the slow EEG waves that it practically cannot be evaluated visually. This question is of special importance because if the same responses occurred during deep slow-wave sleep, the concept of the rebound synchronization response would be valid for the whole SWS process.

The event-related responses of the sleep EEG were first approached by the averaging technique by Williams et al. [12] and Weitzmann and Kremen [11]. The obtained averages in the whole SWS were quite compatible with K-complexes.

Subjects and methods

All-night sleep recordings were obtained under standard laboratory circumstances from 6 healthy subjects, 3 males and 3 females of 21 years mean age. With the exception of the occasional waking states during sleep, combined auditory and light stimuli were presented in a quasi random rate with 20 s average intervals. The stimulus was delivered by a stimulator located 30 cm above the sleeping subject's head. The auditory stimulus was a click of 2000 Hz

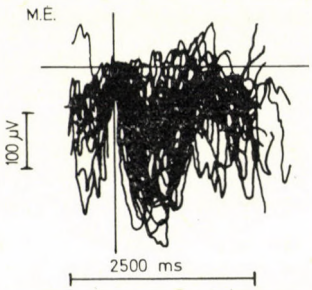

Fig. 1. Superimposed 39 averaged responses recorded during a whole night. The obligatory negative-positive biphasic component appears clearly

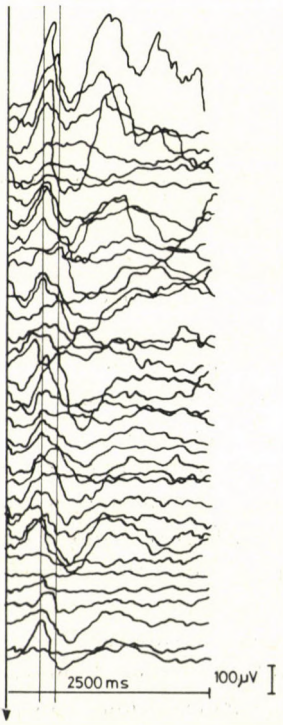

Fig. 2. Averaged responses recorded during a whole night. The first negative peak appears between 450–600 ms (vertical lines)

of 10 W intensity and 10 ms duration; the light stimulus was a white flash of 0.045 Ws. Sleep phases were scored by the criteria of Rechtschaffen and Kales [7]. 2.5 s EEG periods following the stimuli were processed by an averager with 60 point resolution. The sample rate was 16. On the basis of the stimulation frequency, the average of 16 patterns reflected the reactivity of an about 5 min sleep period. Averages containing a sample series overlapping two or more sleep stages were omitted. The highest amplitude of the obligatory components of the averages was measured and considering the rough resolution capacity owing to the low sampling frequency and the long sleep time, the latency time has not been analysed. Analysis of the pattern morphology was made merely to test the presence of the nearly time constant obligatory elements of the averages (Fig. 1).

All averages of an all-night sleep are presented in Fig. 2 to demonstrate these obligatory components in detail. It is obvious that the obligatory part was an asymmetrical biphasic wave with a negative–positive sequence situated between 450–1500 ms and an about 150 ms deviation could be observed in the first negative peak. This obligatory part agreed completely with the components of the signal used by Bremer et al. for automatic pattern recognition of the K-complexes [1].

The amplitude fluctuation of the averages may have resulted from different influences. For example, a decrease of the amplitude could be attributed equally to a real decrease in the amplitude of the samples, to a change in the phase relationships of the response or to an increase in the number of ineffective stimuli. Such a qualitative analysis of the averages has not been aimed at because the biological significance of the three factors is not known and their separate estimation would have had technical limitations. Therefore, not even the absolute numerical values of the amplitudes have been studied, only the trend of amplitude fluctuations was analysed within the given sleep periods.

The hypnograms of the stimulated sleeps did not show any serious differences from the normal nocturnal sleep structure.

Total sleeping time (Mean±S.D.) was	460.7±42.33 min
Stage 1 (+ intermediate sleep)	19.4±13.02%
Stage 2	39.5±13.6 %
Stage 3	12.3± 4.57%
Stage 4	5.2± 3.32%
REM	18.4± 4.42%
Awake	5.2±11.12%

The depth of sleep was somewhat decreased, but from the point of view of the studied problem this change as well as the so-called first night effect was quite indifferent.

Results

In the descending and ascending limbs of sleep cycles in 69% (18 from 26 cases) of the examined sleep stages (minimum 3 averages), and in stage 2 in 74% (14 from 19 cases) there appeared a definite tendency in the amplitude fluctuations, i.e. an increase in amplitude on the descending while a decrease in the ascending limb. Tendencies of fluctuations in the opposite direction were not observed in any of the cases. Figure 3 shows the averages of stage 2 according to the two behaviour types. Beside the averages the part of the hypnogram is also shown.

Within the long deep stages beside the afore-mentioned tendency a micro-fluctuation was also detectable. In stage 2 shown in Fig. 4 within the global increase of amplitude further fluctuations can be observed. The amplitude maximum situated in the middle indicates that the phase shown falls to the deepest part of the cycle.

The synchronization response seemed to be unambiguous even in the deep stages of sleep. Figure 5 shows the averages of a stage 3 and a stage 4 sleep. Apparently in stage 3, inserted between the phases of stage 4, the first and last amplitudes, while in stage 4, forming the deepest part of the cycle, the middle amplitude were representing the peak.

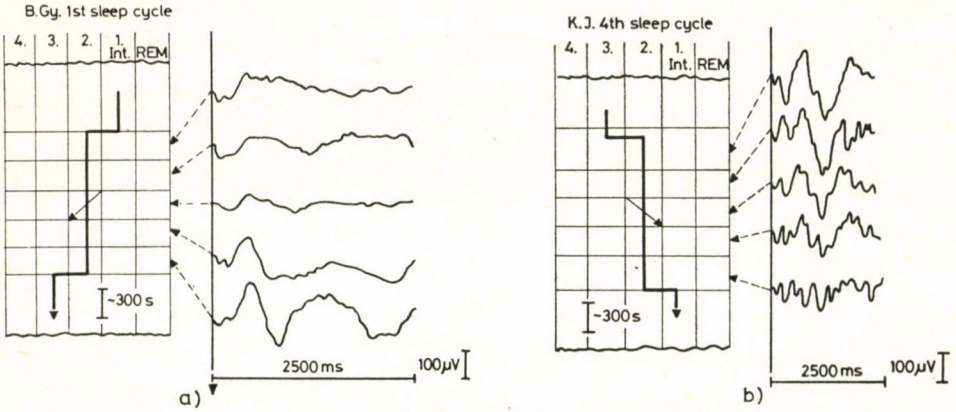

Fig. 3. Averaged responses measured in stage 2. In the descending *(a)* and ascending *(b)* limb of the sleep cycle. On the right part averaged responses, on the left the hypnogram. The arrows in the middle show the part of the hypnogram (about 300 s) in which the average was measured. The arrows on the hypnogram show the actual tendencies of the sleep process. The opposite tendencies can be followed in the change of the amplitudes

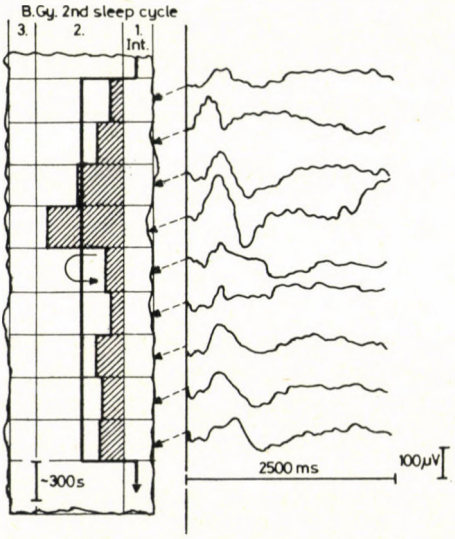

Fig. 4. Averaged responses of a long stage 2. The hypnogram on the left was outlined in detail by an extrapolation from the amplitude values of the averaged responses. Intensive micro-fluctuation of sleep depth during this stage 2. Notations as in Fig. 3

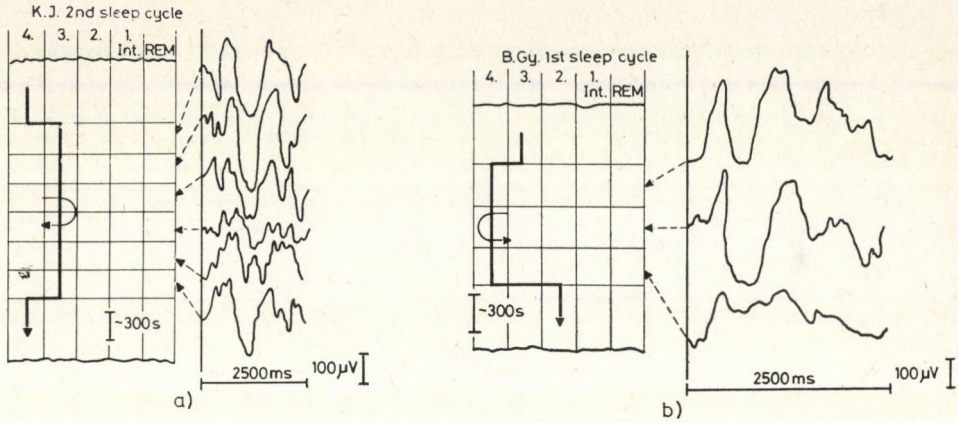

Fig. 5. Averaged responses in stage 3(*a*) and 4(*b*) Notations as in Fig. 3
Fig. 6. Set of averaged responses during a total sleep cycle. Notations as in Fig. 3

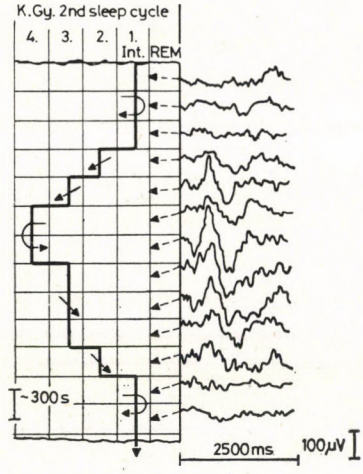

Fig. 7. Averaged responses during a whole night. Notations as in Fig. 3

Figure 6 shows the averages of a comparatively short sleep cycle. Obviously, during the whole SWS the above tendencies of the amplitudes of averages can be followed and the fluctuation seems to be continuous. Figure 7 shows the same in the course of a whole sleep process.

Discussion

The behaviour of the averaged synchronization reaction being the result of a combination of qualitative and/or quantitative electrophysiological changes, it is difficult to find a biological interpretation of the obtained

averages. Church et al. [2] reported on averaging the EEG changes elicited by auditory stimuli in the sleep stages 2 and 3+4. They obtained consequent negative–positive biphasic obligatory averages, corresponding well to our findings. They neglected to discuss the origin of the averages but evaluated the obtained patterns as averaged K-complexes even in the stages of deep sleep (cf. the observations of Weitzmann). The difference between the averages recorded in deep level and those recorded during stage 2 was that beside unchanged obligatory components the averages became tri- or polyphasic. Figures 5*a*, 6*b* and 7 show that the same has been observed in our material. Therefore, the averaged evoked responses of SWS seem to show a continuous transition from the K-complex toward slow-wave groups. The regularity of the obligatory component in the whole SWS is a further evidence of the continuity of the synchronization reaction. In the report of Church et al. the amplitudes of averages seem to be almost equal in stages 2 and 3+4. This is apparently contradictory to the increase of amplitudes parallel to the deepening of sleep, as stressed by us. A detailed analysis of the correlation of amplitudes in different sleep stages shows that the absolute amplitude values were independent of the sleep stages and were dependent only the position of the given sleep stage within the cycle. For example, the amplitudes in stages 2 and 4 could be identical if the given sleep stages had formed the deepest part of its own cycle. (See Fig. 7 showing the amplitudes of stages forming the deepest part of their cycles.)

To sum up, the amplitudes of averages seem to show a uniform behaviour in the whole SWS. The fact that the increase in amplitude implying the deepening of sleep may be followed in the averages of the phasic synchronization reactions elicited by arousal stimuli supports the view that the influence of the arousal system is an essential component of the spontaneous SWS. Consequently, the whole SWS may be based on the response phenomena released by stimuli formed by the reciprocal induction interplay of the sleep–arousal system.

REFERENCES

1. Bremer, G., Smith, J. R., Karacan, I.: Automatic detection of the K-complexes in sleep electroencephalograms. IEEE Transactions on bio-medical engineering **4**, 314–323 (1970).
2. Church, M. W., Johnson, L. C., Seales, D. M.: Evoked K-complexes and cardiovascular responses to spindle synchronous and spindle asynchronous stimulus clicks during N–REM sleep. Electroenceph. clin. Neurophysiol. **45**, 443–453 (1978).
3. Halász, P., Rajna, P., Pál, I., Vargha, M., Kundra, O., Balogh, A., Kemény, A., L'Auné, Gy., Marián, É., Czenner, Zs.: Some electrographic parameters of nocturnal sleep as tracers of the dynamics of the sleep wakefulness system. Recent Developments of Neurobiology in Hungary **8**, 175–209 (1979).
4. Halász, P., Kundra, O., Rajna, P., Pál, I., Vargha, M.: Micro-arousals during nocturnal sleep. Acta physiol. Acad. Sci. hung. **54**, 1–12 (1979).
5. Halász, P., Rajna, P., Pál, I., Kundra, O., Vargha, M., L'Auné, Gy.: Viharos elektrodermográfiás tevékenység mélyalvásban: kísérlet a jelenség magyarázatára (Strong

electrodermographic activity in sleep: experiment for the interpretation of the phenomenon). Magy. Pszichol. Szle **37,** 127–139 (1980).
6. Johnson, L. C., Hanson, K., Bickford, R. G.: Effect of flurazepam on sleep-spindles and K-complexes. Electroenceph. clin. Neurophysiol. **40,** 67–74 (1976).
7. Rechtschaffen, A., Kales, A.: A manual of Standardized Terminology. Techniques and Scoring System for Sleep Stages of Human Subjects. U.S. Government Printing Office Washington, D.C. 1968.
8. Selbach, H.: Reciprocal induction — model of the human sleep process. Ann. N. Y. Acad. Sci. 98–122 (1962).
9. Takigawa, M., Uchida, T., Matsumoto, K.: Cortical potentials time-locked to EKG–R waves during wakefulness and slow wave sleep (stage 2) in the mankind. Brain Nerve **32,** 81–96 (1980).
10. Takigawa, M., Uchida, T., Matsumoto, K.: Correlation between occurrences of spontaneous K-complex and the two physiological rhythms of cardiac and respiratory cycles. Brain Nerve **32,** 127–133 (1980).
11. Weitzmann, E. D., Kremen, H.: Auditory evoked responses during different stages of sleep in man. Electroenceph. clin. Neurophysiol. **18,** 65–70 (1965).
12. Williams, H. L., Tepas, D. I., Morlock, Jr. J. C.: Evoked responses to clicks and electroencephalographic stages of sleep in man. Science **138,** 685–686 (1963).
13. Yamadori, A.: Role of the spindles in the onset of sleep. Kobe J. med. Sci. **17,** 97–111 (1971).

Acta Medica Hungarica, 40 (1), pp. 41–46 (1983)

Immunology

CHARACTERIZATION OF THE EFFECTOR CELLS IN CON A-INDUCED CYTOTOXICITY AGAINST HEp 2 TUMOUR TARGETS

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(Received July 14, 1982)

Con A-induced cytotoxic activity of human lymphocyte subpopulations obtained by cell fractionation procedures was studied in a test system using human epipharynx carcinoma cells (HEp 2) as targets. Only T lymphocytes were cytotoxic, non-T cells exerted no cytotoxic activity, but enhanced the adherence of the tumour cells. $T_{non\text{-}G}$ lymphocytes (Fc-receptor negative T cells) were more active than T_G cells (Fc-receptor-positive T cells) in mediating the Con A-induced cytotoxic reaction.

Keywords: Con A-induced cytotoxicity, human T cells as effector cells, human epipharynx carcinoma (HEp 2) target cells

Introduction

Mitogen-induced cellular cytotoxicity (MICC) has been described as an in vitro model of cell-mediated cytotoxicity [14]. The effector cells lyse their targets in the presence of mitogenic or non-mitogenic lectins [1, 8, 12, 18]. The type of effector cell involved in the process is defined by the origin of target cells (tumour cells, erythrocytes, other cell lines) [1, 7, 16, 18]. Therefore the cells active in different systems are heterogeneous. Only T lymphocytes are capable of mediating PHA- and Con A-induced cytotoxicity against certain tumour targets in the mouse [3, 20, 25] and in man [21, 22, 24]. In addition to T lymphocytes the role of B cells, monocytes, macrophages and polymorphonuclear leucocytes has also been characterized using various sources of erythrocyte targets [4, 5, 7, 20–22, 28, 29].

In man the exact nature of the cell subpopulations participating in the PHA- and Con A-induced cytotoxic reactions remains controversial. Only Fcγ receptor-positive lymphocytes are active in MICC on red blood cells and certain tumour targets [4, 6, 28, 30] in contrast to MICC against other targets where mainly Fcγ receptor-negative cells are the effectors [13, 21]. There is also disagreement concerning the participation of T_M and T_G subpopulations (T lymphocytes/bearing Fc receptors for IgM and IgG) in MICC [17, 24].

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In the present study we have investigated the effector activity of various human peripheral blood mononuclear cell populations in the Con A-induced cytotoxic reaction using human epipharynx carcinoma (HEp 2) target cells.

Materials and methods

Separation of lymphocytes

Peripheral blood mononuclear cells (PBMC) from heparinized venous blood of healthy donors were separated on Ficoll–Uromiro gradient [2]. Viability was checked by trypan blue exclusion.

Fractionation of cells

Phagocytic cells were depleted by carbonyl iron (GAF, USA) treatment. T cells and non-T cells were separated by E-rosette buoyant density gradient centrifugation [10]. Prior to rosette formation, sheep erythrocytes were treated with aminoethylisothiouronium bromide-HBr (Sigma, USA) [15]. After centrifugation, erythrocytes were lysed with distilled water. T cells were further fractionated into Fcγ receptor-positive (T_G) and -negative ($T_{non\text{-}G}$) subsets on EA_{ox}-rosette gradient. Details of the method have been described earlier [11].

Table I

Composition of lymphocyte fractions
(positive cells in per cent; mean ±SEM)

Lymphocyte fraction[1]	Markers			
	E-rosettes	EA-rosettes	B cells	Phagocytes
U	74.5±4.3	16.9±3.2	8.1±1.0	13.1±2.1
T cells	94.1±1.0[2]	21.0±2.8	1.0±0.4	1.3±0.5
T_G	n.d.	71.5±2.2*	n.d.	6.0±2.5
$T_{non\text{-}G}$	n.d.	1.9±0.7	n.d.	1.4±0.7
Non-T	4.3±1.1	33.0±3.7	55.7±8.8	12.2±3.2

U, unfractionated cells after Ficoll separation; T, E-rosette forming cells in the pellet; T_G, Fcγ receptor-positive T cells; $T_{non\text{-}G}$, Fcγ receptor-negative T cells; Non-T, interface of rosette gradient

* Directly counted in the pellet

Purity of lymphocyte fractions was assessed by surface marker studies. T cells were detected by spontaneous (E) rosette formation, Fcγ receptor-positive cells by EA_{ox}-rosette formation, B cells by membrane immunofluorescence, and phagocytic cells by latex particle (Dow Latex, Serva) ingestion as described earlier [11]. The results of cell fractionations are shown in Table I.

Cytotoxicity assay

Cincinnati HEp 2 adherent human epipharynx carcinoma target cells (National Public Health Institute, Budapest, Hungary) were cultured in TC 199 medium supplemented with 10% heat-inactivated calf serum, antibiotics and 25 mM HEPES (Serva, GFR). 24 h prior to the cytotoxicity assay the cells were detached from the plastic surface by 1% trypsin,

washed twice and resuspended in the medium. 100 μl of the suspension containing 2500 viable cells was placed into flat-bottomed microtitration plates (Greiner, GFR) and 0.4 μCi ^{3}H-thymidine (Chemapol, Czechoslovakia) was added. The cultures were then incubated in humidified atmosphere in 5% CO_2 at 37 °C for 24 h to allow adherence of tumour cells to the bottom of the wells. After 24 h incubation, the cells were washed three times with 37 °C medium and 200 μl of the effector cell suspension containing 2.5×10^4 or 5.0×10^4 cells were added giving a 10 : 1 or 50 : 1 effector-target ratio, respectively. Concanavallin A (Con A, Calbiochem, USA) was applied at a final concentration of 25 μg/ml. After a further incubation for 24 h the cultures were washed twice and the remaining cells were frozen at —20 °C. After thawing the cells were harvested using an automated sample harvester (Dynatech, GFR) and the radioactivity of the cells (cpm) was measured. Results were expressed as cytotoxic indices (C.I.) calculated according to the formula:

$$C.I. = -(C_{Con\,A} - C_0)$$

$$C_{Con\,A} = \frac{cpm_{effektor + target + Con\,A} - cpm_{target + Con\,A}}{cpm_{target+Con\,A}} \times 100$$

$$C_0 = \frac{cpm_{effector + target} - cpm_{target}}{cpm_{target}} \times 100.$$

Using this formula positive values represent cytotoxicity, whereas negative ones "stimulation", i.e. enhancement of the adherence of target cells to the plastic surface [26].

Statistical evaluation

Significances were calculated by Student's *t* test.

Results

Role of lymphocyte subpopulations in Con A-induced cytotoxicity

The Con A-induced cytotoxic activity of various human lymphocyte subpopulations on Hep 2 cells is shown in Fig. 1. Ficoll-Uromiro separated human lymphocytes (fraction U) exerted a marked cytotoxic activity (range

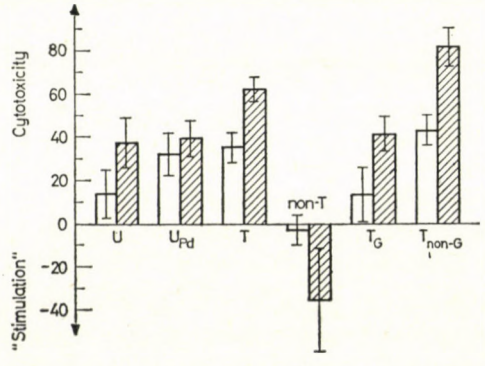

Fig. 1. Con A-induced cytotoxicity of various human lymphocyte subpopulations (mean ± SEM; n=9) Empty columns: 10 : 1 effector-target cell ratio, hatched columns: 50 : 1 effector target cell ratio. U_{Pd}, phagocyte-depleted unfractionated cells. For other abbreviations see Table I. Significances: (50 : 1 effector-target cell ratio). U vs T $P < 0.05$, U vs non-T $P < 0.02$, U vs T_{non-G} $P < 0.002$, U vs T_G and U_{Pd} not significant, T vs non-T $P < 0.002$, T_G vs T_{non-G} $P < 0.01$

−9.8–102% C.I.). Removal of phagocytic cells (fraction U_{Pd}) had no significant effect on the cytotoxicity suggesting that monocytes did not play an important role in this system. Only T cells (fraction T) were cytotoxic, whereas the T cell-depleted lymphocyte subpopulation (fraction non-T) had no activity. Furthermore, this subpopulation promoted survival of the target cells ("negative" cytotoxic index). $T_{non\text{-}G}$ cells were more cytotoxic than the T_G fraction.

Discussion

In spite of extensive studies the nature of the effector cells mediating PHA- and Con A-induced cytotoxicity in man is still a matter of controversy. The purpose of the present study was to investigate the participation of various human effector cell (sub)populations in Con A-induced cytotoxicity, using human HEp 2 target cells.

The results presented suggest that only T lymphocytes are responsible for mediating Con A-induced cytotoxicity against HEp 2 targets. Phagocytic cells and non-T lymphocytes not only lack cytotoxic activity in this system, but they even promote the survival (adherence) of tumour cells. Hence the actual cytotoxic activity of unfractionated mononuclear cells seems to be determined by the counteractions of the cytotoxic T and the tumour cell survival-promoting non-T lymphocytes. $T_{non\text{-}G}$ lymphocytes are more cytotoxic than T_G cells.

Our findings concerning the exclusive role of human T lymphocytes in Con A-induced cytotoxicity against HEp 2 target cells are in good agreement with the data of other authors obtained on tumour targets (P815, EL-4, LP 3, Chang cells) in man [13, 21, 22, 24] and in the mouse [3, 20, 25] as well as on various red blood cell targets [18]. In contrast to systems using other targets in man [6, 7, 21, 22, 29, 30] and in animals [5, 20], non-T lymphocytes and phagocytic cells have no effector activity in the HEp 2 assay. Although carbonyl iron treatment removed more than 98% of phagocytic cells, their possible effector activity cannot be excluded in this system [20].

The mechanism by which non-T cells promote the survival (adherence) of HEp 2 cells is not clear. The phenomenon is similar to the "feeder layer" effect described in connection with the mitogenic or allogeneic stimulation exerted by accessory cells or mercaptoethanol [9, 19, 23]. Similar observations were made by Umetsu et al. on lymphoma cell growth in vitro [27] and by the in PHA-induced cytotoxicity system [26].

Studying the occurrence of Fcγ receptors within the effector cell subpopulations we found that the majority of effector cells was $T_{non\text{-}G}$ lymphocytes in this system. Similar results were obtained by Hersey et al. [13] and Nelson et al. [21] using Chang target cells. Although it is possible that the cytotoxic

activity of the T_G fraction in our experiments was due to contamination with $T_{non\text{-}G}$ cells, the effector activity of T_G lymphocytes themselves cannot be excluded. Other authors found that only Fcγ receptor-bearing cells mediated PHA- and Con A-induced cellular cytotoxicity [4, 6, 28, 30]. Only T_G cells are the effectors against chicken erythrocytes [17], whereas both T_G and T_M lymphocytes are capable of lysing P815 and Chang target cells [24].

It is concluded that only T cells have Con A-induced cytotoxic activity against HEp 2 target cells. Fc-receptor-negative T cells are more cytotoxic than Fc-receptor-positive cells.

Acknowledgements

The authors wish to thank Mrs. M. Schender and Mrs. J. Czikovszky for skilful technical assistance.

REFERENCES

1. Asherson, G. L., Ferluga, J., Janossy, G.: Nonspecific cytotoxicity by T cells activated with plant mitogens in vitro and the requirement for plant agents during the killing reaction. Clin. exp. Immunol. **15,** 573–589 (1973).
2. Böyum, A.: Separation of leucocytes from blood and bone marrow. Scand. J. clin. Lab. Invest. 21. Suppl. **97,** 77–89 (1968).
3. Bonavida, B., Bradley, T. P.: Studies on the induction and expression of T cell-mediated immunity. V. Lectin-induced nonspecific cell-mediated cytotoxicity by alloimmune lymphocytes. Transplantation **21,** 94–102 (1976).
4. Bonavida, B., Robins, A., Saxon, A.: Lectin-dependent cellular cytotoxicity in man. Transplantation **23,** 261–270 (1977).
5. Britton, S., Perlmann, H., Perlmann, P.: Thymus-dependent and thymus-independent effector functions of mouse lymphoid cells. Comparison of cytotoxicity and primary antibody formation in vitro. Cell. Immunol. **8,** 420–434 (1973).
6. Cordier, G., Samarut, C., Revillard, J.-P.: Contribution of lymphocytes bearing Fcγ receptors to PHA-induced cytotoxicity. Immunology **35,** 49–56 (1978).
7. Cortens, M., Sklar, S., Richter, M.: The detection and identification of subpopulations, monocytes and neutrophils capable of effecting a mitogen-induced cell-mediated cytotoxic reaction towards erythrocytes of various species. Immunology **41,** 623–634 (1980).
8. Gately, M. K., Martz, E.: Comparative studies on the mechanism of nonspecific Con A-dependent cytolysis and specific T cell-mediated cytolysis. J. Immunol. **119,** 1711–1722 (1977).
9. Geier, S. S., Cresswell, P.: Monocytes as accessory cells in the human mixed lymphocyte response: lack of requirement at high cell densities. Cell. Immunol. **49,** 190–195 (1980).
10. Gergely, P., Bakács, T., Cornain, S., Klein, E.: Fc receptors on human blood B lymphocytes. Clin. exp. Immunol. **28,** 99–102 (1977).
11. Gergely, P., Ernberg, I., Klein, G., Steinitz, M.: Blastogenic response of purified human T-lymphocyte populations to Epstein–Barr virus (EBV). Clin. exp. Immunol. **30,** 347–353 (1977).
12. Green, W. R., Ballas, Z. K., Henney, C. S.: Studies on the mechanism of lymphocyte-mediated cytolysis. XI. The role of lectin-dependent cell-mediated cytotoxicity. J. Immunol. **121,** 1566–1572 (1978).
13. Hersey, P., Edwards, A., Edwards, J.: Characterization of mononuclear cells in human blood. Clin. exp. Immunol. **23,** 104–113 (1976).
14. Holm, G., Perlmann, P., Werner, B.: Phytohaemagglutinin-induced cytotoxic action of normal lymphoid cells on cells in tissue culture. Nature (Lond.) **203,** 841–843 (1964).
15. Kaplan, M. E., Clark, C.: An improved rosetting assay for detection of human T lymphocytes. J. immunol. Meth. **5,** 131–135 (1974).

16. Kirchner, H., Blaese, R. M.: Pokeweed mitogen-, concanavalin A- and phytohemagglutinin-induced development of cytotoxic effector lymphocytes. An evaluation of the mechanisms of T cell-mediated cytotoxicity. J. exp. Med. **138,** 812–824 (1973).
17. Lum, L. G. Muchmore, A. V., Decker, J. M., Blaese, R. M.: MICC cytotoxic effector function of human T lymphocyte subpopulations bearing Fc-receptors for IgG and IgM. Clin. exp. Immunol. **37,** 558–561 (1979).
18. MacDermott, R. P., Nash, G. S., Saint, J. G., Clark, E. A., Zaras, A. G., Boldt, D.: Autologous human cellular cytotoxicity induced by mitogenic and non-mitogenic lectins. J. Immunol. **117,** 1402–1404 (1976).
19. Miller, T., Stewart, E.: "Filler" cells are non-immunological cushions. Cell. Immunol. **49,** 307–316 (1980).
20. Muchmore, A. V., Nelson, D. L., Kirchner, H., Blaese, R. M.: A reapprasial of the effector cells mediating mitogen-induced cellular cytotoxicity. Cell. Immunol. **19,** 78–90 (1975).
21. Nelson, D. L., Bundy, B. M., Pitchon, H. E., Blaese, R. M., Strober, W.: The effector cells in human peripheral blood mediating mitogen-induced cellular cytotoxicity and antibody-dependent cellular cytotoxicity. J. Immunol. **117,** 1472–1481 (1976).
22. Nelson, D. L., Bundy, B. M., West, T. D., Strober, W.: The nature of the effector cells mediating mitogen-induced cellular cytotoxicity (MICC) and antibody-dependent cellular cytotoxicity (ADCC). Cell. Immunol. **23,** 89–98 (1976).
23. Persson, U., Hammarström, L., Möller, E., Smith, C. I. E.: The role of adherent cells in B and T lymphocyte activation. Immunol. Rev. **40,** 78–101 (1978).
24. Pichler, W. J., Gendelman, F. W., Nelson, D. L.: Fc receptors on human T lymphocytes. II. Cytotoxic capabilities of human Tγ, Tμ, B and L cells. Cell. Immunol. **42,** 410–417 (1979).
25. Sawada, J., Osawa, T.: Characterization of lectin-induced cellular cytotoxicity mediated by mouse spleen cells and role of lymphotoxin. Immunology **41,** 525–534 (1980).
26. The, T. H.: PHA-induced cytotoxicity of human lymphocytes. J. immunol. Meth. **19,** 29–39 (1978).
27. Umetsu, D. T., Lerman, S. P., Thorbecke, G. J.: Accessory cell requirements for lymphoma growth in vitro and in irradiated mice. Cell. Immunol. **42,** 139–154 (1979).
28. Waller, C. A., Campbell, A. C., MacLennan, I. C. M.: Two populations of lymphocytes involved in phytohaemagglutinin-induced cytotoxicity of a dividing target cell. Scand. J. Immunol. **5,** 931–939 (1976).
29. Wislöff, F., Fröland, S. S.: Studies on the cytotoxic activity of human lymphoid cells activated by concanavalin A. Int. Arch. Allergy. **45,** 456–466 (1973).
30. Wislöff, F., Fröland, S. S., Michaelsen, T. E.: Characterization of subpopulations of human lymphoid cells participating in phytohaemagglutinin and concanavalin A-induced cytotoxicity. Int. Arch. Allergy. **47,** 488–497 (1974).

Acta Medica Hungarica, 40 (1), pp. 47—49 (1983)

Laboratory

A NEW METHOD FOR MEASUREMENT OF H_2 IN EXHALED AIR IN THE PRESENCE OF PERMANENT GAS MIXTURES

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(Received June 14, 1982)

A new instrument has been devised for the measurement of H_2 in human expired air. The device is simple in construction and can substitute gas chromatography (GC) in differential diagnosis, clinical dietetics, pharmacological research, environmental and labour safety analysis etc., at low cost.

Keywords : measurement of H_2, exhaled air, permanent gas mixtures, malabsorption syndrome, labour safety, soda-lime filter

Introduction

An increase in the quantity of H_2 in expired air is a sensitive parameter in some gastrointestinal diseases and/or malfunction of enzymatic systems. Exact knowledge of the amount of H_2 in expired air offers important information concerning differential diagnosis, clinical research, monitoring the recovery of patients with malabsorption syndromes, etc. [2].

Today, gas chromatography is the most widespread method for the measurement of H_2 concentration in permanent gas mixtures [1, 2, 3, 4].

For this special purpose some chromatographic detector systems have been developed, but at high price and high operating costs. This fact has made us to devise a new and cheap instrument for measuring H_2 content in human expired air.

Materials and methods

The patients breathe during 1 to 5 min in a 5 l closed system where the $O_2 : N_2$ ratio is 5 : 3 at zero time. The CO_2 gas is removed by a soda-lime filter. The device is calibrated in a simple way was using different quantities of standard H_2. Figure 1 shows the device schematically and Fig. 2 the detector sign in relation to the calibrating H_2.

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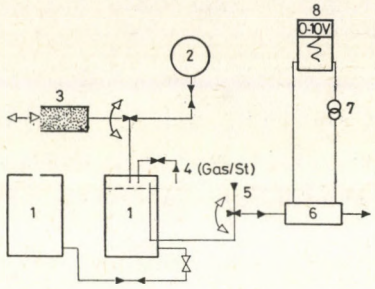

Fig. 1. Schematic picture of the instrument for the measurement of H_2 gas. 1 = hydrodynamic shock absorbers; 2 = pneumatic shock absorbers; 3 = CO_2 absorber; 4 = H_2 inlet (St); 5 = shunt; 6 = converter–detector system; 7 = power supply; 8 = recorder

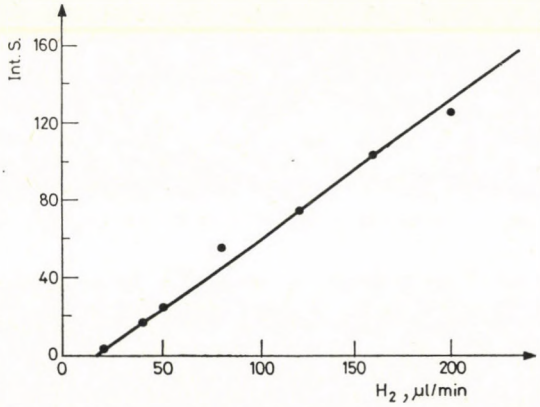

Fig. 2. Detector signs versus H_2 (breathing time 5 min). Sensitivity: 500 p.p.b. 5 min^{-1} 5 l^{-1}. Reproducibility (five measurements) = 5%; it depends on the reproducibility of volume measurements and methods of integration. Linearity: the detector signs are linear against H_2 concentrations from 100 p.p.b. to 40 p.p.m. min^{-1}

Results and discussion

The advantages of the device against those of GC are its simple structure, and low operating and original cost, the last one being less than 1% of that of GC. The sensitivity of measurement is shown in Fig. 2 where H_2 of less than 20–30 μl has no biological importance. Another advantage is that O_2 and other gases must not be removed while GC analysis depending on detectors and columns requires total removal of O_2 and CO_2 and sometimes of water, too.

Our studies revealed that the instrument is able to measure other gases (hydrocarbons and other organic vapours) too, in intermittent or continuous operation after adequate development of the converter and detector.

REFERENCES

1. Bergman, I., Coleman, J. E., Evans, D.: Gas chromatograph with an electrometrical detector for the measurement of hydrogen and carbon monoxide in parts per million range, applied to exhaled air. Cromatographia **8,** 581–583 (1975).
2. Bodánszky, H., Soutter, V., Leleiko, N.: Lehelet H_2 meghatározás mint diagnosztikus eszköz szénhidrát rendellenességben (Measurement of H_2 in exhaled air as a means of diagnosis of carbohydrate malabsorption). Orv. Hetil. **122,** 2039–2040 (1981).
3. Bond, J., Lewitt, M. D.: Use of pulmonary hydrogen (H_2) measurements to quantitate carbohydrate absorption. J. clin. Invest. **51,** 1219–1225 (1972).
4. Lewitt, M. D., Donaldson, R. M.: Use of respiratory hydrogen (H_2) excretion to detect carbohydrate malabsorption. J. Lab. clin. Med. **75,** 937–945 (1970).

Acta Medica Hungarica, 40 (1), pp. 51—57 (1983)

EFFECT OF TRITON X-100 ON THE ELECTROPHORETIC MOBILITY OF THE CREATINE KINASE ISOENZYMES OF SERUM AND TISSUES

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(Received December 10, 1981)

The distribution of creatine kinase isoenzymes was studied in homogenates of human skeletal muscle, myocardium and brain tissue, and in sera.

For the study of the cytosolar isoenzymes (MM, MB, BB) the tissues were homogenized with a Ca^{++}- and Mg^{++}-containing buffer. The membrane-associated isoenzymes were solubilized with buffer containing 1% Triton X-100. The isoenzymes were separated on polyacrylamide gel slabs.

After treatment with Triton X-100 a membrane-associated isoenzyme of high activity, corresponding in mobility to cytosolar MM, was identified in brain tissue. No similar fraction was demonstrable in muscle tissue. The difference in the results is attributed to differences in the sensitivity of the creatine kinase isoenzymes of muscle and brain tissues.

The cytosolar MM fraction of human sera is of more anodal mobility than that of tissue homogenates, and in the MM region subfractions have been demonstrated in some cases. Triton X-100 did not affect the electrophoretic mobility of the creatine kinase isoenzymes of the sera, or of the tissue homogenates.

Keywords: creatine kinase, isoenzymes, electrophoresis

Introduction

According to earlier studies [5], the non-ionic detergent Triton X-100 affects the creatine kinase (CK) activity of homogenized human muscle brain tissue and of sera in different ways. The CK activity of skeletal muscle was found to decrease, this effect being less distinct in the case of heart muscle. On the other hand, the activity of brain tissue showed a significant increase. None of the sera activities showed changes of this kind.

The changes in activity under the effect of the detergent were attributed to differences in composition and sensitivity of the isoenzymes of the individual tissues. It is under this aspect that the cytosolar and membrane-associated isoenzymes of these tissues have been studied.

According to our studies, Triton X-100 has not modified the electrophoretic mobility of these isoenzymes. The type MM isoenzyme was character-

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istic of muscle tissue and serum, but its electrophoretic mobility and homogeneity greatly differed in muscle and serum and the latter revealed more MM subfractions.

Materials and methods

Human tissues. Specimens were taken from the rectus abdominis muscle, the left ventricular myocardium and the cerebral cortex within 36 h post mortem. The tissues were stored at +4 °C and processed within 2 h. The studies were aimed at the demonstration of cytosolar (MM, MB, BB) and of membrane-associated (mitochondrial, synaptosomal, myosin-bound, etc.) isoenzymes. The tissues were therefore processed parallel in two different buffers. The first isolating medium was an isotonic Ca^{++} and Mg^{++}-containing Tris-NaCl buffer pH 7.2 (TNCM) [1]; by its use it was thought to minimize damage to the intracellular organelles and thus to study prevalently cytosolar CK. The other buffer (II) contained 1% Triton X-100 in addition to the components of buffer I. On the evidence of earlier data [5] and later results, 1% Triton X-100 is capable of solubilizing membrane-associated proteins, while leaving the CK activity unaffected. This allowed to measure cytosolar and membrane-associated activities together in Triton X-100-containing samples. The method has been described in detail earlier [5].

Supernatants of the tissue specimens obtained at 30,000 *g* were used for studies of CK activity, the measurement of proteins, and for polyacrylamide gel electrophoresis.

Human sera. Normal sera and sera with abnormally increased activity were studied for CK isoenzymes, in all cases within 24 h of withdrawal. Before electrophoresis the total protein content of the sera was also determined.

Measurement of proteins was done in detergent-free supernatants by the method of Lowry et al. [15], in Triton X-100-containing samples according to Chi-Sun Wang and Smith [4] by the use of Na-lauryl sulphate, and in the sera by the biuret method.

Measurement of CK activity. CK activity was estimated on the basis of the method of Oliver [19] and Rosalki [20] by means of the UV test with glutathione as –SH activator, as prescribed by the manufacturers (CPK-activated test, Boehringer–Mannheim Co. Cat. No. 124174).

Separation of CK isoenzymes. For the separation of the isoenzymes from the supernatants and the sera stored at +4 °C polyacrylamide gel electrophoresis was performed on the first and third day after isolation (ORTEC Electrophoresis System, Model 4100).

The polyacrylamide gel slabs were prepared according to Blomberg and Burke [2].

Sample-free pre-electrophoresis. Mesurement of CK activity [19, 20] requires the auxiliary enzymes hexokinase and glucose-6-phosphate dehydrogenase (G-6-PDH). These auxiliary enzymes reach by diffusion only the superficial layer of the gel therefore, Sjövall and Jergil used electrophoresis to bring them into the gel [23]. Since these auxiliary enzymes move slower than does the most mobile CK (BB), they require sample-free electrophoresis to gain access to the gel. For this purpose the Tris buffer (Reanal, Budapest), pH 8.5, 0.01 mol/l (μ-0.075) and glycine (Reanal, Budapest) 0.05 mol/l, were used as solvents.

To the cathode buffer 500 U/l hexokinase and 100 U/l G-6-PDH (both Boehringer, Mannheim) were added. Electrophoresis was done at 100 V and 25 mA per plate, for 30 min. Subsequently the cathode buffer was poured off and set aside.

The anode buffer contained no auxiliary enzymes.

Preparation of samples. Before electrophoresis the samples were mixed with 40% saccharose 1 : 1, so as to make the contact zone with the cathode buffer sharper and to avoid mixing of the samples. The 20–40 μl aliquots transferred from the samples contained 0.8 to 20 μg protein, the serum samples 120 to 160 μg protein.

Electrophoresis of samples. CK isoenzyme electrophoresis was carried out in the cathode and anode buffers described under pre-electrophoresis. Separation was started at 100 V and 25 mA and continued 10 min later at 200 V and 50 mA/plate for 1 h. The conditions of electrophoresis were modified in some cases, the run being prolonged for 4 to 6 h (70–80 V, 15 mA/plate). This provided for a sharper separation in some of the isoenzyme zones. Electrophoresis was performed at room temperature, but the initial temperature of the buffer was +4 °C. In view of the light sensitivity of CK the electrophoresis dish was covered with a dark cloth [26]. On completion of separation the gel plates were removed from the cells and placed into Tris HCl buffer (0.01 mol/l, pH 7.0, +4 °C). In this manner optimal reduction of the pH of the plates, which had been imbided with the buffer of pH 8.5, was attained prior to detection.

Detection of CK activity. The gel plates were transferred into glutathione-activated CK test solution, prepared according to the instructions of the manufacturers (Boehringer–Mannheim), and incubated at 37 °C for 45 to 60 min.

CK activity was detected partly photographically on the basis of the fluorescence of $NADPH_2$ formed during the reaction, partly by subjecting the gel to a histochemical reaction with p-nitrotetrazolium blue.

Results

Isoenzymes of MM mobility were detected by polyacrylamide electrophoresis in skeletal muscle homogenates of MM and MB mobilities in heart muscle, and of BB and MM mobilities in brain tissue (Fig. 1). The isoenzyme patterns and staining intensities of the skeletal muscle and myocardial tissues, whether treated with TNCM (I) or with Triton X-100-containing TNCM (II), were found to be identical. On the other hand, the fraction of MM mobility of brain tissue treated with Triton X-100 showed a more intensive fluorescence and staining than the same fraction of the sample treated with Triton-free TNCM. Addition of Triton X-100 prior to electrophoresis to the supernatants of the muscle and brain tissues treated with Triton-free buffer, failed to affect the intensity of fluorescence and staining. The isoenzyme activities were not quantified.

In normal sera exclusively isoenzymes of MM mobility were found. The MM band of the sera was more anodal in mobility than that of the tissue homogenates (Fig. 1) moreover, it was not invariably homogeneous. CK–MM entering the circulation in vivo (i.e. endogenous CK) was more than once found to be inhomogeneous after the first 60 min of electrophoresis (Fig. 1),

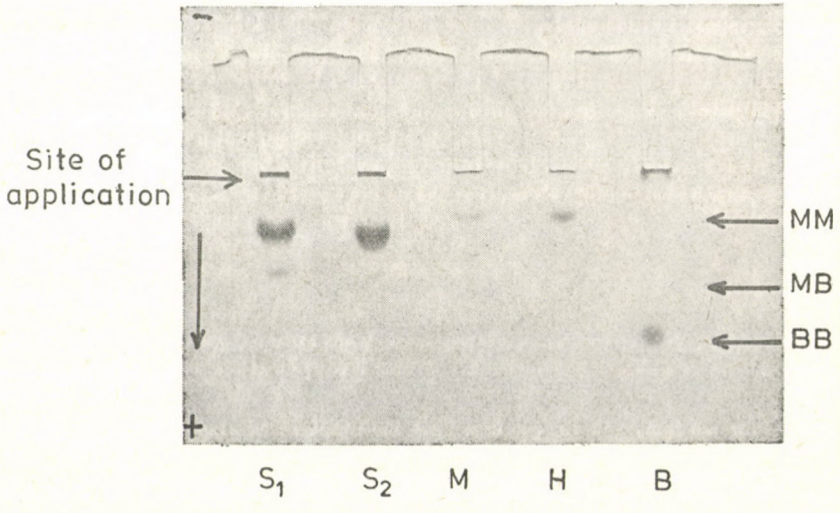

Fig. 1

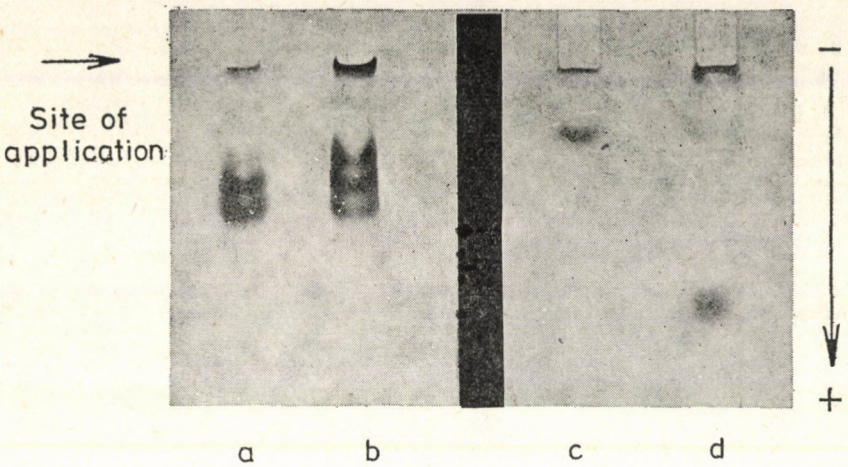

Fig. 2

but if it was run for 4 to 6 hours, subfractions were as a rule demonstrable and the findings reproducible (Fig. 2). In this case the CK–MM region generally showed 2 to 3 isoenzyme bands. The individual sera differed in the number and staining intensity of the subfractions. The pathological sera of high CK activity usually contained 3 subfractions in the MM region (Fig. 2). Triton X-100 left the distribution, mobility and staining intensity of these isoenzymes unchanged.

If for the separation of the isoenzymes a gel containing 0.01% Triton X-100 had been used, the isoenzymes, regardless of the detergent content of the samples, stained more intensively with tetrazolium blue. On the other hand, the detergent-containing gel failed to intensify the $NADPH_2$-fluorescence.

Discussion

According to the present findings, Triton X-100 did not affect the qualitative CK isoenzyme pattern of muscle tissue. In skeletal muscle cytosolar isoenzymes of MM mobility in heart muscle isoenzymes of MM and MB mobilities were found. On the other hand, in the cerebral tissue not only CK–BB, regarded as the only cytosolar fraction, could be demonstrated, but also a cerebral isoenzyme of MM mobility which, in samples treated with Triton, showed an increased staining and fluorescence (Fig. 1). Mitochondrial CK was observed cathodally from cytoplasmic MM by Sax et al. [22] and Somer et al. [25] and anodally by Jacobs et al. [10]. Friedhoff and Lerner [6] and Wevers et al. [32], on the other hand, described membrane-associated isoenzymes having the same mobility as cytoplasmic MM. Since in brain tissue treated with Triton X-100 we have also found a significant CK–MM fraction,

we are justified in regarding it to be membrane-associated. This may account for the increased CK activity of the Triton-treated brain samples.

The MM region of the muscle specimens processed with Triton showed no significant difference in intensity; it only contained isoenzymes of cytoplasmic mobility (MM, MB). We thus failed to confirm the presence of membrane-associated isoenzymes in homogenized muscle tissue. The present results seem to be consistent with our earlier observations [5] according to which the tissue homogenates treated with Triton X-100 showed an increased protein concentration and little, if any, change in CK activity, whereas in brain tissue treated in the same manner the increase in protein concentration was accompanied by a considerably increased enzyme activity.

The present findings thus suggest that the CK isoenzymes of the two different tissue homogenates are different in sensitivity [6, 7, 12, 17, 24, 27] and this would account for our failure to demonstrate membrane-associated isoenzymes in the Triton treated muscle tissue in contrast with the similarly treated brain tissue [3, 6, 10, 11, 21, 30]. Inactivation in the course of Triton treatment is practically irreversible. In our earlier studies we were indeed unable to increase the CK activity of muscle tissue homogenates, in opposition to brain tissue homogenates, to any significant extent by the use of -SH–activators [5].

CK activity and electrophoretic mobility of the human sera remained unaffected by Triton X-100 [22]. On the other hand, the MM band of the sera was of faster mobility than that of the tissue homogenates, regardless of the use of Triton, and in some cases it was inhomogeneous (Figs 1, 2). CK serum isoenzymes of "atypical electrophoretic mobility" have been described by other authors as well [8, 14, 22, 34]. Sjövall and Jergil [23] and Smith [24] have also found 2 to 3 subfractions in the MM region of some pathologic sera. Wevers et al. [31, 32] attributed these findings to postsynthetic modifications of the M subunit. The electrophoretic mobility of CK isoenzymes may be modified as a result of its linkage to β-lipoproteins [29] or to immunoglobulins [13, 16, 28] in vivo, but changes in vitro may also be produced in the course of storage for several days [1, 9, 33]. We have also noted such changes in some instances. The postsynthetic modifications of CK are not merely of theoretical interest. The frequency of their occurrence on the one hand, and the increasing use of isoenzyme studies, on the other, may involve major diagnostic problems [18, 28].

REFERENCES

1. Bayer, P. M., Gergely, T. H., Gabl, F., Deutsch, E.: Serum-induced changes in electrophoretic mobility of creatine phosphokinase isoenzymes? Clin. chim. Acta **79**, 261–263 (1977).
2. Blomberg, D. J., Burke, M. D.: Isoenzymes of creatine kinase; separation by acrylamide gel electrophoresis. Ann. clin. Lab. Sci. **4**, 456–461 (1974).

3. Booth, R. F. G., Clark, J. B.: Studies on the mitochondrially bound form of rat brain creatine kinase. Biochem. J. **170,** 145–151 (1978).
4. Chi-Sun Wang, Smith, R. L.: Lowry determination of protein in the presence of Triton X–100. Anal. Biochem. **63,** 414–417 (1975).
5. Feledi, É.: Detergens, hőmérséklet és tárolási idő hatása a szöveti és szérum kreatinkináz aktivitásra (Effects of detergent, temperature and time of storing on the activity of serum and tissue creatine kinase isoenzymes). Kísérl. Orvostud. **34,** 124–130 (1982).
6. Friedhoff, A. J., Lerner, M. H.: Creatine kinase isoenzyme associated with synaptosomal membrane and synaptic vesicles. Life Sci. **20,** 867–874 (1977).
7. Gowda, K. S., Roberts, R., Sobel, B. E.: Detection of myocardial infarction with serum CPK isoenzymes in surgical patients. Circulation **50,** Suppl. III. 109 (1974).
8. Homburger, H. A., Jacob, G. L., Forsman, R. W., Beckala, H. R.: Clinical laboratory experience with metastable creatine kinase MM. Clin. Chem. **25,** 1101 (1979).
9. Hyong, CH. W., Meltzer, H. Y, Joung, J. I., Goode, D.: Effect of incubation in human plasma on electrophoretic mobility of brain-type creatine phosphokinase. Clin. chim. Acta **73,** 257–265 (1976).
10. Jacobs, H., Heldt, H. W., Klingenberg, M.: High activity of creatine kinase in mitochondria from muscle and brain and evidence for a separate mitochondrial isoenzyme of creatine kinase. Biochem. biophys. Res. Comm. **16,** 516–521 (1964).
11. James, G. P., Harrison, R. L.: Creatine kinase isoenzymes of mitochondrial origin in human serum. Clin. Chem. **25,** 943–947 (1979).
12. Jockers-Wretou, E., Pfleiderer, G.: Quantitation of creatine kinase isoenzymes in human tissues and sera by an immunological method. Clin. chim. Acta **58,** 223–232 (1975).
13. Jockers-Wretou, E., Plessing, E.: Atypical serum creatine kinase isoenzyme pattern caused by complexing of creatine kinase-BB with immunoglobulins G and A. J. clin. Chem. **17,** 731–737 (1979).
14. Ljungdahl, L., Gerhardt, W.: Creatine kinase isoenzyme variants in human serum Clin. Chem. **24,** 832–834 (1978).
15. Lowry, O. H., Rosenbrough, N. J., Farr, A. L., Randall, R. J.: Protein measurement with the Folin phenol reagent. J. biol. Chem. **193,** 265–275 (1951).
16. Meyer, I. J., Thompson, J. A., Kiser, E. J., Haven, G. T.: Observation of a variant creatine kinase isoenzyme in sera and breast tumor cytosols. Amer. J. clin. Path. **74,** 332–336 (1980).
17. Nealon, D. A., Henderson, A. F.: Stability of commonly used thiols and of human creatine kinase isoenzymes during storage at various temperature in various media. Clin. Chem. **23,** 816–829 (1977).
18. Nemesánszky, E., Bayer, P. M.: A kreatinkináz- (CPK)-izoenzimek klinikai jelentősége (The clinical importance of creatine kinase isoenzymes). Orvostud. Akt. Probl. **29,** 109–128 (1977).
19. Oliver, I. T.: A spectrophotometric method for the determination of creatine phosphokinase and myokinase. Biochem. J. **61,** 116–122 (1955).
20. Rosalki, S. B.: An improved procedure for serum creatine phosphokinase determination. J. Lab. clin. Med. **69,** 696–705 (1967).
21. Saks, V. A., Chernousova, G. B., Voronkov, I. I., Smirnov, V. N., Chazov, E. I.: Study of energy transport mechanism in myocardial cells. Circulat. Res. **35,** Suppl. III. 138–149 (1974).
22. Sax, S. M., Moore, J. J., Giegel, J. L., Welsh, M.: Further observations on the incidence and nature of atypical creatine kinase activity. Clin. Chem. **25,** 535–541 (1979).
23. Sjövall, K., Jergil, B.: Coupled enzyme reactions in polyacrylamide gels: Isoenzymes of serum ATP: creatine phosphotransferase. Scand. J. clin. Lab. Invest. **18,** 550–552 (1966).
24. Smith, A. F.: Separation of tissue and serum creatine kinase isoenzymes on polyacrylamide gel slabs. Clin. chim. Acta **39,** 351–359 (1972).
25. Somer, H., Uotila, A., Konttinen, A., Saris, N. E.: Creatine kinase activity and its isoenzyme pattern in heart mitochondria. Clin. chim. Acta **53,** 369–372 (1974).
26. Thomson, W. H. S.: An investigation of physical factors influencing the behaviour in vitro of serum creatine phosphokinase and other enzymes. Clin. chim. Acta **26,** 105–120 (1969).
27. Tsung, S. H.: Creatine kinase isoenzyme patterns in human tissue obtained at surgery. Clin. chem. **22,** 173–175 (1976).
28. Urdal, P., Landaas, S.: Macro creatine kinase BB in serum, and some data on its prevalence. Clin. Chem. **25,** 461–465 (1979).
29. Velletri, K., Griffiths, W. C., Diamond, I.: Abnormal electrophoretic mobility of a creatine kinase MM isoenzyme. Clin. Chem. **21,** 1837–1838 (1975).

30. Vial, C., Font B., Goldschmidt, D., Gautheron, D. C.: Dissociation and reassociation of creatine kinase with heart mitochondria; pH and phosphate dependence. Biochem. biophys. Res. Comm. **88,** 1352–1359 (1979).
31. Wevers, R. A., Wolters, R. J., Soons, J. B. J.: Isoelectric focusing and hybridisation experiments on creatine kinase (EC 2.7.3.2.). Clin. chim. Acta **78,** 271–276 (1977).
32. Wevers, R. A., Delsing, M., Klein Gebbink, J. A. G., Soons, J. B. J.: Post-synthetic changes in creatine kinase isoenzymes (EC 2.7.3.2.). Clin. chim. Acta **86,** 323–329 (1978).
33. Wevers, R. A., Mul-Steinbusch, M. W. F. J., Soons, J. B. J.: Mitochondrial CK (EC 2.7.3.2.) in the human heart. Clin. chim. Acta **101,** 103–111 (1980).
34. Yuu, H., Takagi, Y., Seniu, O.: Creatine kinase isoenzyme of high molecular mass in serum of a cancer patient. Clin. Chem. **25,** 1190–1191 (1979).

Acta Medica Hungarica, 40 (1), pp. 59—72 (1983)

Book reviews

BÁNÓCZY, Jolán: *Oral Leukoplakia.* Akadémiai Kiadó, Budapest and Martinus Nijhoff Publishers. The Hague–Boston–London 1982. 231 pp. with 125 figures + 17 colour photos, 62 tables, Ft. 200.—

A very timely topic is dealt with in this monograph by Professor Bánóczy who has been engaged in the study of lesions of the oral mucous membranes for nearly thirty years at the Oral Medicine Clinic of the Department of Stomatology and Oral Surgery, University Medical School in Budapest, headed for the most time by L. Sugár who was the initiator in Hungary of systemic follow-up studies in these patients. In these decades Bánóczy became an expert of this topic, well-known over the world, having been elected to membership in the WHO Collaborating Centre for Oral Precancerous Lesions.

Oral leukoplakia is the term to describe a white patch of the oral mucosa that cannot be removed by rubbing, and is not classifiable as any other oral disease. Accordingly, in the monograph, besides leukoplakia all other mucosal lesions are thoroughly elucidated that are to be excluded when diagnosing leukoplakia; such as oral lichen, leukoedema (white sponge naevus), Candida infection, morsicatio buccarum et labiorum. These lesions are dealth with first of all in reliance on clinical experience but the discussions are duly supported by conventional histological, exfoliative cytological, electron microscopical–ultrastructural, and even morphometric stereological studies. Patients observed at any time, in whatever stage of the condition were followed up more or less regularly through as many years as proved feasible; mostly through an interval long enough to assess changes in appearance, micromorphological features and/or other accessible parameters. Having considered the anamnestic data as well as all information obtained from the patients as to their actual and former habits, diet, beverages, preceding dental treatment, other systemic and infectious diseases, factors responsible aetiologically were unequivocally shown, first of all smoking, mechanical irritation, additionally "hard drinks" electric potential differences, and finally inflammatory and atrophic changes of the oral mucous membranes including syphilis. The clinical studies allowed to differentiate between three distinct types of leukoplakia, viz. simple, verrucous, and erosive, that were shown to differ micromorphologically and to be of quite different proneness to turn into carcinoma. It was shown unequivocally that the lesions of leukoplakia are capable of changing both as to extent and to type, ad melius as well as ad peius. Improvements can be induced by eliminating the exciting factor(s), and occasionally by proper conservative treatment. Nonetheless, as a last resort of therapy, surgical excision of the lesions is advocated, particularly in types markedly prone to change to carcinoma. — As the central problem of clinical relevance, the conversion of leukoplakia (and other white lesions) into cancer is strongly emphasized through the whole treatise, practically non-existent in the simple type, and frequent with the erosive form. The author also demonstrates her profound acquaintance with the pertinent international literature as well as her ability of assessing critically the reported results of studies by others. Under these circumstances, it was justifiedly expected from both the medico-dental community and the publisher that the result be a first class reference monograph.

If one tries, however, to delve deeper into the study of the book, some shortcomings of different importance are obvious. Some are probably due to a lack of proper editing. So in Figs 43–45 one cannot see the arrows that are mentioned in the explanatory texts; in Figs 54, 56, 88 characters are shown in the illustrations without explaining what they mean; in Figs 113 and 117 arrows are seen that are not mentioned in the legends. The construction of some tables is objectionable. In Table 35, some column heads are erroneous; what is printed as "Totals/Men" really shows the sum of female patients in the different age groups, whereas what is presented as "Totals/Women" are really the summed counts of male plus female patients. In Table 45, the headings "Cytological findings" and "Histology" are interchanged. In the lowest row of the column "Normal leukoplakia" (?) instead of *3* observations, Figure *2* is correct. Table 30 is misconstructed: the subheading "Type change" under "Improved cases" should be completed to "Type change from", and accordingly, under "Spread" the subheading should be changed to "Progressive type change into". Then, in column 2 of "Improved cases" instead of *47* in the first horizontal row, *7* should stay in the second, and *40* in the third one, in agreement with the text. That the cipher *8* is a misprint in the 1st row of the 4th column, the correct one being *4*, is easily detected. — A more logical order of the columns in Table 31 would considerably facilitate its understanding, viz. "Total No. of leukoplakia (1), Total No. of lesions showing type change (2), Type change in % (3), Regressive lesions (4), and Progressive lesions (5)".

The usual form of presenting statistical significance figures is not always adhered to. In Table 12, the explanations are correct but the p values for females are shown without preceding $<$ signs as 0.05, and 0.02, respectively, such signs standing correctly in the lower part of the Table before the analogous p values for men. On p. 21, $0.6 < p > 0.3$ is printed, but if $p > 0.6$, it is necessarily > 0.3. On p. 36, the lacking statistical significance of a difference is shown by $p > 0.5$. In Tables 5 and 36, including the explanatory text on p. 76, instead of generally accepted terms (expected and observed values), other ones are used. As to demonstrating statistical significance of the age dependences, the quotients $\frac{\text{observed}}{\text{expected}}$ are tabulated instead of having used the common χ^2 test.

Of more concern to the reviewer were inconsistencies of the numerical figures, both as to base numbers and as to percentages, differences being obvious in some instances between the tabulated figures and those relied upon in the discussions. A few examples: in Table 7, 12 carcinomas of the tongue are shown, and in Table 6, 44 patients with lingual leukoplakia; the text on p. 23 states, however, that of 31 cases of lingual leukoplakia 12 turned into cancer. No mention is made what happened to the other 13 patients of Table 6. — Similarly, 30 cases of leukoplakia are tabulated in the floor of the mouth, and 3 cases of carcinoma at this location, but the rate of malignant transformation of the former into the latter is given as 9.7% in the text on p.27. — In the caption of Table 11, "Counts of keratinized cells. . ." is printed, the summarized numbers being exactly 100 in 10 boxes, but 82 and 125, respectively, in the remaining two ones. It cannot be understood that exactly 100 was the average cell count in 10 instances, but deviant numbers in two. — The sex distribution of patients with migratory glossitis is given as 24:46 in Tables 23 and 24, but as 25:45 in Table 25.

Serious objections are to raised against the presentation of the results of exfoliative-cytology in the diagnosis of oral carcinoma (Tables 45 and 46, pp. 93–97). As a non-invasive method it was enthusiastically welcomed by clinicians but was soon proven — as in this monograph too — to be unreliable, but this conclusion is neglected in the text. The figures of Table 45 indicate that of 24 cases classified as "malignant" by cytology, 4 were false positive findings (after correction of a misprinted figure), i.e. 16.67% (although not more than 1.99% of all 201 examinations). In contrast, of 25 invasive and 4 in situ carcinomas demonstrated histologically, 5 were classified by cytology as plain leukoplakia and 4 additional ones

as "atypic" (suspect of carcinoma). The 9 cytologically misdiagnosed cases are 31% false negative results, and excluding the atypic cases, more than 17% remain. In relation to the total number of cytological findings the frequency figures were 4.43, and 2.49%. The frequency figures of false cytological diagnosis to be relied upon in assessing the usefulness of the method in the diagnosis of oral carcinoma are 17 and 31 (17) per cent far higher than tolerable. Hereupon no impact is exerted from percentage frequencies of coinciding cytological and histological findings referred to on p. 94. Breaking up the comparison of the two methods according to the clinical type of leukoplakia, shown in Table 46, has no additional informational value either; in contrast to 82 tabulated findings in erosive leukoplakia, in the text 81 cases are referred to; apparently the tabulated figure being correct.

The reviewer was perplexed by the different size of the population mentioned in different places of the book; 520 leukoplakia patients are referred to on p. 15, with 31 conversions to malignancy; in contrast to 500 leukoplakias diagnosed clinically, 48 having been shown histologically as carcinoma, on p. 72.

Having shown some defects of the monograph, some of its merits should be emphasized. One is the quality of the illustrations. Of a total of 142, only one was found of poor quality (Fig. 46 on p. 95); it is too dark to show what should be seen. Another merit is the clarity in expression and style. As to this item, only two objections are raised. In the column heads of Tables 45 and 46, and in the text on p. 143 "normal leukoplakia" is written what in itself is a contradiction. With some phantasy, the reader with be aware of that here simple leukoplakia and normal oral mucous membrane are meant, respectively. — Contradictory opinion is expressed as regards the turning into carcinoma of median rhomboid glossitis on p. 59 to the statement of p. 57 that in the author's longitudinal studies such a change was not observed in any case. — Chapter IX is an excellent summary — suitable as a reliable guide for the dentist how to proceed with lesions of the oral mucous membranes.

Counterbalancing the merits of the monograph and its shortcomings, paying due attention to the author's high international reputation, in spite of its defects the book may turn to a success with the perspective of a second edition. In this, the inconsistencies should, and could be avoided probably easily.

† P. Adler

Z. Antalóczy, I. Préda (eds): *Electrocardiology '81.* Akadémiai Kiadó, Budapest 1982. 620 pp. Ft 560

The steady progress in every field of medicine brings forth a profusion of clinical and experimental evidence. The progress in cardiology due to rapid advance in heart surgery and in cardiodiagnostic methods is a case in point. It is hardly possible to keep abreast with the steadily accumulating information. Textbooks of cardiology are more or less obsolete by the time of their publication, and the literature is too vast to be kept in evidence. The best help is to have in print the subject-matter of congresses or meetings on various topics of cardiology. The success of such books depends on two questions: whether they are published in good time and whether they contain really up-to-date material. By these standards this book may be regarded as a success and it fills a real need. It is based on the proceedings of the 8 International Congress of Electrocardiology held in Budapest 1–4 September 1981. Its main subject is the progress of electrocardiology but fortunately various other aspects of cardiology also receive excellent coverage. Remarkable papers were read on electrophysiology, in particular on the development and problems of cellular microelectrophysiology and on recent methods of investigation. The experimental and clinical studies of cardiac arrhytmias also belong to the main subjects of the Congress. We find several essays of high interest on arrhythmias

consequent upon heart surgery, caused by lymph retention in the heart and on the arrhythmic property of the heart. Drug therapy of arrhythmia was also on the agenda. Within the central subject, i.e. the progress of electrocardiology, computerization, mathematical models, experimental modelling of the electric field of the heart received particular attention. Lectures were given on the recent methods of ECG-recording such as surface potential mapping, electrovector cardiography, etc. as well. The extensive use of echocardiography in association with other special methods is brought into due prominence.

Electrocardiology '81 is a rich source of up-to-date information. Its layout and production are faultless. It will be of value to heart surgeons, physiologists and pharmacologists. Those who have special interest in electrocardiography, cardiac arrhythmias, and experimental cardiology will find it indispensable.

F. SOLTI

L. SURJÁN, Gy. BODÓ (eds): *Borderline Problems in Otorhinolaryngology*. Proceedings of the XIIth World Congress of Otorhinolaryngology, Budapest, Hungary, 21–27 June 1981. Akadémiai Kiadó, Budapest 1982

The view that international congresses are out of date has been finding strong support lately. In fact, the representatives of the various specialities are steadily increasing in number and there is a relentless process of subspecialization in all branches of medicine. For attendants of a world congress with thousands of participants even personal meeting with colleagues, let alone fruitful exchanges of ideas, may be impossible. Organization of meetings confined to some sphere of lesser scope may be found more convenient. Though this is certainly true, yet, in the words of Professor Surján, president of IFOS, we have to do our best to prevent the science of otorhinolaryngology (ORL) from falling apart. It is, therefore, desirable to take stock of its current status from time to time.

Though the world congresses may fall short of this ideal, we have no better alternative. There is nothing that can make up for personal contact, even if it means renouncing of a parallel program. But then, anyone can have the material in print, provided it is published in good time. It is, therefore, to be welcome that the proceedings of the round-table conferences of the last World Congress of ORL have appeared with little delay.

In accordance with the borderline problems of ORL on which the discussions of all conferences had been centred, the material is arranged into chapters as follows. Ophthalmological and neurological aspects of rhinology; oncological problems of ORL; current problems of paediatric ORL; immunological aspects; traumatology and ORL; present possibilities of diagnosis; management and prevention of hearing defects; advances in phoniatrics. In two further chapters we find remarkable accounts of current trends and of recent results concerning the pathophysiology of ORL. The closing chapter deals with education and qualification, questions which have thus far failed to receive due attention.

It is to be regretted that some of the reports, having arrived after the deadline, could not be inserted.

The production of the book does credit to the book publishers but the printing technique fails to reproduce all shades of the photographs, particularly of the micrographs, with the usual fidelity.

For the obvious limitation of the number of participants and the missing views of some authorities, the book offers the compensation of collecting information from all parts of the world. The volume will not fail to achieve its aim to serve as a source of information stimulating further studies and thus to contribute to the progress of ORL.

M. BAUER

H. D. KAUFMAN (ed.): *The Haemorrhoid Syndrome.* Abacus Press, Kent, England 1981. 157 pp. £ 17.5

The interest of the book lies in the fact that more than 50% of the world's population over 50 years is more or less severely affected by haemorrhoids. This book, written by a distinguished team of authors, provides relevant information on the subject to proctologists, general surgeons, and also to physicians interested in this branch of medicine.

The chapter by W. H. F. Thomson sums up traditional knowledge and recent evidence relating to the morphology and pathology of haemorrhoids. He gives particular attention to the anal pads enclosing arterio-venous shunts. These are actually physiological structures, but knowledge of their changes is essential to modern therapeutic approach. All this also applies to the chapter on anal physiology (B. D. Haucock) which provides important clues to the selection of the appropriate intervention. The next chapter, written by the Editor is of prime clinical relevance; it discusses the history and the clinical symptomalogy. The distinguishing features of mucosal and vascular type haemorrhoids are pointed out and the complications (thrombosis, strangulation, ulceration, infections) determining the lines of therapy are discussed. A separate chapter (J. L. Carven) is devoted to high-residue diets which, though certainly no panacea, are none the less important factors of prevention and therapy. A further chapter by the Editor is on injection therapy, which accounts for 50 to 70% of the first interventions in his country. It is primarily recommended for vascular type haemorrhoids in stage I–II. In the chapter on the indications and technique of strangulation with the rubber ring according to Barron (M. R. B. Keighley) the procedure is claimed to be rewarding as it has cured 62% of the patients. In the chapters on forced dilatation aimed at overcoming the hyperfunction of the sphincter regarded as an aetiological factor, and on lateral sphincterotomy, the authors, D. B. Haucock and M. R. B. Keighley, formulate the indication of these interventions with some caution, and fail to convince the reader of the justification for their routine use. In the sections on surgical management and its details H. D. Kaufman describes the technique of cryosurgery and emphasizes its benefits, but points to its failure in cases of any major prolapse. In the next chapter P. Schofield states that the aims of a successful operation, whether of open or closed type are, in addition to mobilization of the internal haemorrhoids from the sphincter, a high ligation of the vessels with maximal preservation of the mucosa and removal of the involved skin.

Haemorrhoids of mucosal type associated with skin tags cause circular prolapse and call for elective surgery. Acute interventions may be successful in case of thrombosed, strangulated haemorrhoids. Proper selection of the cases and adequate familiarity of the surgeon with the technique being taken for granted, minor interventions are highly gratifying and allow to avoid major surgery in a fair proportion of the cases.

The book provides an authoritative and up-to-date account of the current surgical methods and on the general lines of therapy. In the reviewer's opinion it is unsurpassed by any other work published on the subject in recent years.

M. KUN

Vaccination Certification Requirements for International Travel and Health Advice to Travellers 1981. WHO, Geneva 1981. 65 pp., 2 tables, 1 appendix, 3 maps, Sw. Fr. 18.—

These annual publications of identical title and layout are very useful. The present volume provides information essential to international travel.

The first part deals with three important and timely issues subjected to WHO regulations. In these years no international vaccination certificate ought to be required in any country,

with the exception of one for yellow fever. Regrettably, the WHO regulations to this effect are disregarded in many countries. For instance, preventive vaccination against cholera is required in various parts of the world. This has harmful consequences since cholera vaccinations are of limited value and may, therefore, give the vaccinated person a false sense of safety from infection, which may interfere with the necessary epidemiological measures including early diagnosis, observation, isolation, etc. Some countries still require immunization against smallpox, though this has been abolished. On the other hand vaccination against yellow fever has to be strictly observed where it is justified. The vaccination remains effective for 10 years from the 10 day of its application. Revaccination should be undertaken still within this time before immunity provided by the previous vaccination has vanished. The vaccination certificate is valid for 10 years. A smallpox vaccination certificate is acceptable only if it is in compliance with the international form found in the Appendix of the book. There exists no vaccination against malaria, only regular preventive antimalarial medication. This alone provides safety to the individual, but it has to be borne in mind that, despite continuous attempts at eradication, malaria under given circumstances may be of utmost gravity, even fatal. WHO must, therefore, have reports from the respective countries on the prevailing malaria situation in good time, with a view to preparing a collective account intended for publication.

Section 2 lists alphabetically the vaccination certificate requirements and the malaria situation in the individual countries; 63 of the 195 listed here have no such requirements. In the remaining countries a vaccination certificate is required for the following diseases: yellow fever 96, yellow fever + cholera 31 countries, yellow fever + cholera + smallpox 1 country, yellow fever + poliomyelitis 1 country, cholera 2 countries. Malaria risk and/or occurrence has been reported from 102 of the 195 countries.

Section 3 groups the travellers' health hazards on the basis of the following categories: environmental (climatic and meteorological) factors and ill-effects; food and drinking water; arthropodes and hazards of other kinds. The section closes with an outline of the medical tasks after the traveller's return. The physician finds here very useful tabulated recommendations on his tasks if a patient seeks his advice for fever, diarrhoea, etc. after return from a foreign country.

Section 4 groups the hazards according to continents and countries, but in closer detail than the former section.

Section 5 points to the possibilities of prevention, starting with the basic principles of personal and specific prevention. The excellent text directed at the essentials is backed by two useful tables. The first presents the antimalarial drugs (name, dosage, use and adverse effects), the second the diseases related to food and drinking water (name, pathogenic agent, route of transmission, geographic prevalence). The names, regions and countries are indexed at the end of the book.

The appendices include a printed vaccination certificate form and 3 maps on the malaria situation and the prevalence of yellow fever in Africa and South America.

L. Cselkó

Vaccination Certificate Requirements for International Travel and Health Advice to Travellers 1982. WHO, Geneva 1982. 70 pp., 2 tables, 1 appendix, 3 maps, Sw. Fr. 12.—

This volume is similar in layout to the previous one published in 1981. Here, too, information on the international vaccination certificates and the pertaining questions are centred on the following issues: requirements in various countries; risk factors; grouping of risks according to continents and countries; suggestions for prevention. The present publica-

tion, much to its advantage, has five more pages, for the greatest part on prevention. Moreover it is cheaper than its predecessor.

In the list of vaccination certificate requirements 195 countries are represented; 61 countries have no such requirements as opposed to the other 134. On the basis of diseases we find the following distribution: yellow fever 97 countries, yellow fever + cholera 31 countries, yellow fever + cholera + smallpox 1 country, yellow fever + poliomyelitis 1 country, yellow fever + typhoid 1 country, cholera 2 countries. Since the previous year vaccination certificate requirements had thus come into force in two more countries (vaccination for yellow fever and for yellow fever + typhoid, respectively). The booklet contains, in addition, accounts of the malaria situation from 102 countries.

L. Cselkó

G. Edwards, A. Arif: *Drug Problems in the Sociocultural Context.* Public Health Papers No. 73. WHO, Geneva 1980. 258 pp., 9 figures, Sw. Fr. 18.—

This study has two major and closely related aims. The first is to review the sociocultural aspects of drug-taking. The second is to present guidelines for the formulation of policy and the planning of programmes.

Chapter 1 gives an account of the activities which are pursued by the international agencies in close cooperation with WHO all over the world in relation to the drug problems. Chapter 2 on sociocultural patterns of drug use, gives a long series of dramatic images in the form of case-studies from Thailand, Malaysia, India, Pakistan, Burma, Andean region of South America, Mexico, USA, Jamaica, Egypt and Kenya. It provides information on the most widely used drugs such as opiates, heroine, cannabis, coca, marijuana, etc. and on the distribution of users by age, sex, occupation and geographic region. The differences and similarities of drug use in the various countries are dealt with in Chapter 3. In this context the use of LSD and of other types of drug-dependence are considered and problems of multiple-drug use are discussed.

It is likewise on the basis of case studies and examples that Chapter 4 entitled "Variety in Health Care Approaches", illustrates the withdrawal practices in the different countries, for instance withdrawal programmes for addicts on a voluntary basis in Hong Kong withdrawal treatment on a religious basis in Buddhist temples in Thailand (against a compensation corresponding to $ 1.0 a day), detoxification of opium addicts in Sri Lanka, activities of withdrawal treatment in emergency hospital units (!) in London, withdrawal therapy for alcocholics based on the traditional religious and cultural background in Japan. The role of the general practitioner is given due prominence in this chapter.

Chapter 5 shows in what way countries differ in their treatment of drug problems. It takes a sober look at the situation and admits with praiseworthy sincerity that certain problems involved by cultural influences have remained unsolved to the present day.

Chapter 6 gives a survey of the strategies for reducing demand for drugs, on the basis of case reports. In the first part the world-wide problems posed by psychotropic drugs in particular by diazepine-derivatives are pointed out under their general aspects. This is followed by a detailed account of the situation in those countries in which abuse of alcohol and smoking add to the problems of drug-dependence.

Chapter 7 discusses the "Prevention and the Balance of Strategies"; it provides a conceptual framework and offers practical guidelines. Chapter 8 sets down the basis for policy design, programme planning and points to the sociocultural perspectives in the first place at a nation-wide, governmental level. Chapter 9 entitled "The Sense of the Future", though not the longest, but certainly one of the most interesting parts of the book, points to future

trends on the ground of estimates, predictions, taking into consideration changes in sociocultural patterns, drug problems and relevant measures, specific and nonspecific treatment, prevention, studies of the background and international cooperation. Though this chapter is oriented somewhat theoretically, its approach is realistic and sober, therefore, it is likely to be of help in the planning of projects aimed at the solution of the drug problem.

L. CSELKÓ

Assessment of Public Health and Social Problems Associated with the Use of Psychotropic Drugs. Technical Report Series No. 656. WHO, Geneva 1981. 54 pp., Sw. Fr. 4.0

A WHO Expert Committee meeting was held in Geneva, on 15–20 September 1980, with the purpose of examining and evaluating the implementation of the Convention on Psychotropic Substances (1971). The subject-matter of the meeting is presented in this booklet.

Chapter 1 introduces the subject by a survey of the problems posed by the psychotropic substances and of the regulations concerning their use. Chapter 2 on the technology for assessment of extent of use of psychotropic substances discloses remarkable facts. While in the U.S.A. the consumption of stimulants and barbiturates has been found to decline, minor tranquillizers and/or sedatives exceed in consumption all other drug classes. While the overwhelming majority of users of tranquillizers, hipnotics and sedatives on medical prescription are females, those of the same drugs dispensing without medical prescription are males. In Europe, too, conpsumption of barbitures and stimulants has been declining in parallel with increasing demands for nonbarbiturate hypnotics, particularly for benzoiapines. In the U.K. 5% of the female and 3% of the male population used tranquillizers for more than 6 months in the last year. In other countries of Western Europe too, the use of psychotropic drug has been increasing. In Australia old-age pensioners accounting for 9% of the general population consume approximately 45% of the marketed psychotropic substances. In the Philippines, Thailand and Malaysia stimulants, sedatives of hallucinogens are being used by 4 to 8% of the secondary school students. In the same chapter useful guidelines are offered for the assessment and quantification of consumption of psychotrophic substances. Some particular aspects are pointed out and recommendations are given.

Chapter 3 on the techniques for the assessment drug-related health problems, outlines these methods, evaluates their practicability, points out the relationships between the consumption of psychotrophic substances and morbidity and mortality, emphasizes the role of toxicological analysis, and gives some recommendations on this issue.

The techniques for the assessment of drug-related social problems form the subject of Chapter 4. Here, too, the present situation is regarded as the practicability of the methods, consideration being given to behavioural problems, criminal behaviour and to socioeconomic effects.

Chapter 5 sums up the conclusions and gives general recommendations.

There ar three annexes: *(i)* WHO's activities in relation to its international treaty obligations; *(ii)* the role of nongovernmental organizations; and *(iii)* proposed mechanisms for the monitoring of psychotrophic drugs.

The book ends with a comprehensive list of reference.

L. CSELKÓ

P. HORNBY, D. K. RAY, P. I. SHIPP, T. L. HALL: *Guidelines for Health Manpower Planning*, WHO, Geneva 1980. 368 pp., Sw. Fr. 26.—

This volume covers the subject-matter of a course on health manpower-planning. The course consisted of 12 sessions, accordingly it is presented in 12 chapters: 1. Health Manpower Planning; 2. Ten Steps to Health Manpower Planning; 3. Health Manpower Situation Report on Existing Manpower and Services; 4. Future Supply of Manpower; 5. Manpower Requirements — Quantity, Quality and Distribution; 6. Mismatches Between Supply and Requirements; 7. Solving Mismatches; 8. Organizational and Management Problems; 9. Manpower Strategy and Outline Plan; 10. Detailed Health Manpower Development Plan; 11. Implementation and Monitoring; 12. Concluding Session.

The actual subject-matter is introduced by general information on the course itself. There are two printed forms, one for the particulars of the student (name, age, education, employment, sphere of activity), the other for the opinion he has formed on the course (evaluation, comments, suggestions, etc.).

A clear arrangement, an excellent formulation of the text, instructive tables and illustrative diagrams belong to them erits of the book. Workers in developing as well as in industrialized contries will find it a reliable source of practically rather than theoretically oriented information.

L. CSELKÓ

M. SCHAEFER: *Intersectoral Coordination and Health in Environmental Management;* Public Health Papers No. 75. WHO, Geneva 1981. 122 pages, 5 tables, 4 figures, Sw. Fr. 9.—

The two decades elapsed between 1950 and 1970 witnessed a definite orientation of the public mind all over the world toward human environment and its impact on health and on socioeconomic values. Environmental preservation began to rank with economic and social development as a major objective of public policy. The first chapter of the booklet "Background to the Study", gives definitions, historic facts and points to various aspects. At the end of this chapter we find a country report as a preliminary model and comments on it. The 11 conditions or provisions that should characterize such a model are specified in full detail.

The "model" consists of statements specifying 11 conditions or provisions that should characterize a proper or adequate national system of coordination, recognizing that no country has yet fully developed such conditions and provisions. In summary terms, the 11 provisions are, (a) active concern for health and environmental values on the part of the politically effective segment of the population; (b) reflection of such concern among political and managerial, leaders; (c) institutionalized arrangements for intersectoral study, communication and decision-making at policy and technical levels; (d) mechanisms for the development, enunciation, and dissemination of guiding policies, laws, and norms; (e) linkage of these mechanisms with the organs of political power; (f) systematized and updated information on problems, solution technology, policies, and programmes; (5) coordinated scientific/technical capacity for determining and forecasting conditions and risks.

Chapter 2 gives a "Description and Comparison of National Structures and Processes"; it summarizes information on the structures and major processes in 21 countries or territories at the time of reporting in 1978. 19 of the 21 countries — the two exceptions being Iran and Iraq — may be classed on the basis of geographic, demographic and socioeconomic features to the following 5 clusters: 1. Canada, Federal Republic of Germany, Japan, United States of America; 2. China, Czechoslovakia, Poland, Yugoslavia; 3. Denmark, Hong Kong, New Zealand; 4. Chile, Egypt, Mexico, Turkey; 5. India, Nepal, Pakistan, Sri Lanka.

Chapter 3 bears the title "Factors Influencing Intersectoral Coordination". It develops the former lines further by evaluating the various factors in the operational and monoperational systems and by giving detailed directives on intergovernmental coordination and on the role of the public health system.

The conclusions are found in Chapter 4 the results achieved in intersectoral coordination are surveyed further requirements are pointed out and an account is given of the active measures which have been taken.

Appendix 1 contains information on each of the 21 countries, Appendix 2 presents an analysis of explanatory variables.

L. Cselkó

R McMahon, E. Barton, M. Piot: *On being in Charge.* WHO, Geneva 1980. 366 pp., Sw. Fr. 12.—

The joint WHO–UNICEF Committee has provided invaluable help by this book for the solution of the urgent problems posed by the organization of primary health care first of all in the developing countries.

Chapter 1, an introduction, outlines the background, objectives, methods and design of the study. It also provides brief information of comparative character on the countries participating in these activities.

Chapter 2 is entitled "Decision-Making for PHC: Evolution of the Approach as a Process", it discusses questions of theoretical orientation, taking certain countries as models. In conclusion it states that the primary health care approach has evolved as a process in response to a continuing need, exemplified by the historical evolution in the study countries. From the present analysis of the events decision-making has emerged as a process operating under the influence of interacting factors which may be grouped into four types: internal political commitment; external influences; non-governmental initiatives; and health sector and government planning processes. Some of these factors are dealt with in subsequent chapters.

Chapter 3, "Politics, Government and Planning", is of very wide scope, pointing to important fundamental aspects, considerations, and providing practical guidelines relating to the strategy of public health development. It also gives recommendations concerning economic tasks, suprastructural decisions, the decentralization of these activities, on bureaucracy, etc.

In Chapter 4 the possibilities of community involvement and of intersectional cooperation are dealth with. Chapter 5 deals with the question how decisions on primary health care turn out in practice. The substance of Chapter 6 is implied in its appropriate title, "Decisions on Health Sector Resources: a Litmus-test of Commitment". Finally, Chapter 7 sets down the recommandations of the Joint Committee on Health Policy in eight points.

Though the booklet has been primarily intended for use in developing countries, the practical information it offers will be helpful to organizations of any other country having to cope with primary health care tasks.

L. Cselkó

National Decision-Making for Primary Health Care. WHO, Geneva 1981. 69 pp., Sw. Fr. 12.—

This is a most appropriate guide for middle-level management in primary health care. It lends itself to teaching as well as to learning purposes. Its style is clear, unambiguous. From the didactic point of view it also deserves every praise. Its logical arrangement, its illustrations

and sketches make even intricate subjects accessible to the learner. Its line-drawing and diagrams are very instructive. There are 5 sections.

Section 1 provides an answer to the question: What is management? Here we find definitions and descriptions of the various functions of management. This part closes with exercises of prevalently test character.

Section 2 is entitled "Working with People in the Health-Team Approach". The subject of basic importance is discussed under the following aspects: What is a health team? How to lead a health team activities and controlling and assessing work. This is likewise followed by exercises.

Section 3 "Managing Resources" deals with managing equipment, drugs, money, time space paperwork and closes with exercises. This is one of the most extensive, but at the same time one of the most interesting chapters with illustrations, tables and sketches of high didactic value written in a clear, easy style.

Section 4 on "Planning Primary Health Care Services" falls into 3 chapters on planning, implementing and evaluating health activities, respectively. This is the other most successful section of the book with a masterful formulation of the tasks. It also ends with exercises.

The Glossary at the end of the book is most useful. Its definitions are concise and accurate.

L. CSELKÓ

International Digest of Health Legislation. Vol. 31. No. 4. WHO, Geneva 1981. 312 pp.

This is one of the quarterly publications of WHO in which the regulations, instructions and decrees issued by the health legislation of each Member State are currently registered.

The regulations of drug abuse in developing countries is the subject of Section 1. It offers information of high interest, derived from the situation in 32 countries. It registers the regulations which have been hitherto in force, relating to the compounds classified as drugs, to the definition of drug dependence, to treatment, rehabilitation and to ways and means of international cooperation.

Section 2 contains the regulations issued by the national government of 16 countries (Bermuda, Bulgaria, Canada, Chile, Denmark, Ecuador, Greece, Hong Kong, Hungary, Lesotho, Libyan Arab Yamahiriya, Luxemburg, New Zealand, Turkey, United Republic of Tanzania, U.S.A.). Accurate title, finding list and brief summary of the regulations are given for each country.

In Section 3 we find statutory provisions, measures and declarations which have come into force by the association of several countries, on a strictly regional basis (Association of South East Asian Nations, European Communities, Mediterranean Coastal States, Mexico and the United States of America, South America, United Nations Economic Commission for Europe.)

Section 4 covers some recent problems concerning practically all continents. We find here succint information concerning smoking, alcoholism, environmental protection, sanitary problems of food and drinking water.

From this book it emerges that the health regulations of various countries are directed mainly at drugs, smoking, occupational hazards, environmental pollution, epidemiological questions and vaccination, though more than fifty other health aspects are also considered.

L. CSELKÓ

BECHTEL, R. M.: *Low-Cost Rural Health Care and Health Manpower Training.* Vol. 7. An annotated bibliography with special emphasis on developing: International Development Research Centre, Ottawa 1981. 142 pp.

This book, the 7th volume of the widely known, useful series, is of similar outlay and extent as its precedessors and contains 700 items of information on five main issues in a condensed form.

Section 1 lists the basic sources, the reference works, Section 2 (perhaps the most interesting part), covers questions of organization and planning. In the latter part the summaries of the studies on staffing, organization and management projects, cultural and financial aspects, geographic distribution of services, epidemiology, family planning, mother and child welfare and nutrition are arranged into 7 chapters. Section 3 on primary health care implementation, is shorter. It falls into 4 chapters covering the following topics: rural patient care, mobile units and services, community health education. Section 4 on primary health manpower training and utilization, gives sources of definitely practical orientation, those relating to professional and nonprofessional workers being on the whole grouped apart. In this section teaching aids are also referred to. Section 5 includes formal evaluative studies.

There are very good lists of abbreviations and of acronyms. The terms and geographic names are indexed.

L. CSELKÓ

Drinking-Water and Sanitation, 1981–1990. WHO, Geneva, 56 pp., 2 tables, Sw. Fr. 9.—

In order to improve the health of the populations a programme of the International Drinking Water Supply and Sanitation Decade (to be referred to as Decade) has been elaborated by WHO and is presented in this book.

Chapter 1 gives a survey of the Decade targets and global coverage, subsequently it outlines the factors of the Decade approach. These are, at the national level: 1. complementary sanitation and water-supply development; 2. strategies that give precedence to underserved populations, both rural and urban: 3. programmes that will serve as a model for self-reliant, self-sustaining action; 4. use of socially relevant system that people can afford; 5. association of communities in all stages of projects; coordination of water supply and sanitation programmes with those in other sectors; 7. association of water supply and sanitation with health improvement.

At the international level emphasis is laid on 1. promoting and supporting national programmes for the Decade through technical cooperation; 2. concentrating technical cooperation on building up national capacities and generating dynamic, self-sustaining programmes; 3. promoting technical cooperation among developing countries; and 4. encouraging the external financing of the national Decade activities.

Chapter 2 on community-water supply and sanitation in the Decade, raises a diversity of problems, mainly on a conceptual basis (shortcomings of governmental strategies and of international support; pertinent conferences, resolutions and conventions; WHO policy). This chapter also deals with topics of more practical orientation relating to the essential elements of Decade (complementary water supply and sanitation development, conditions in urban and rural areas, practicable programmes, participation of the community). Chapter 3, the most homogeneous of all, gives recommendations to the governments of the individual countries in a framework for national action: coordinating and action committees should be brought into existence for the duration of the Decade; planning should be of continuous character and social participation should be secured for the entire plan from its very design

to its execution. Strategic seminars, educative work and good connections are essential. In the practical guidelines for the programmes, emphasis is laid on the questions of the coverage programmes, the programmes and of their equilibrium. In this context the programmes for the rural areas, urban-fringe areas, small- and medium-sized towns and for areas of regional scope also receive thorough consideration. Questions of manpower, mass communication media, health education, participation of the community, technology, information and supervision are also discussed.

There are two appendixes: 1. Resolutions and action programme of the United Nations Water Conference (Mar del Plata, 14–15 March 1977); 2. Resolution 35/18 of the General Assembly of the United Nations.

L. Cselkó

Disability Prevention and Rehabilitation. WHO, Geneva 1981. 40 pp., Sw. Fr. 3.—

This booklet gives an account of the Expert Committee Meeting on Disability Prevention and Rehabilitation held in Geneva on 17–23 February 1981, in the from of a lucid, informative summary directed at the essentials.

Chapter 1 is of introductory character. Chapter 2 gives definitions of terms and concepts related to the disability process and its rehabilitation. Though each definition is clear and explicit, there are three which deserve particular notice, owing to their practical importance. In the context of health experience, an *impairment* is any loss or abnormality of psychological, physiological or anatomical structure or function; a *disability* is any restriction or lack (resulting from an impairment) of ability to perform an activity in the manner or within the range considered normal for a human being; and *handicap* is a disadvantage for a given individual, resulting from an impairment or a disability, that limits or prevents the fulfilment of a role that is normal for that individual depending on his age, sex and social and cultural factors. This chapter gives the main lines of approach to disability prevention and rehabilitation at a social level and to the social integration of the disabled. The interest of the issues raised in Chapter 3 is implied in its title: "Review of Prevalence of Disability, Quality of Life among the Disabled and Future Trends in Prevalence", but the situation revealed by its figures is anything rather than favourable. On the evidence of statistics from 1976, the proportion of disabled, handicapped, underprivileged persons in the world was estimated at 10%, the number of disabled children at 60 to 70 million in 1970. Should the existing preventive facilities not be exploited and further developed, this number may attain 135 to 150 million by the year 2000. Chapter 4 deals with the approach to the problem of disability and its strategy. The specific strategy may be summed up as follows. *(i)* Prevention of disability through all types of measure within and without the health sector, that contribute to a reduction in the incidence of impairment. If an impairment is present, measures should be taken to reduce its severity or to postpone the occurrence of disability and handicap. *(ii)* Provision of rehabilitation, using the primary health care approach. Community-based rehabilitation services with an appropriate system of supervision and referal should be provided, with the aim of total coverage of all populations. These services deliver at least the most essential care, and form an integral part of the national socioeconomic development programme. The necessity for the earliest possible general approach and specific strategy is emphasized.

Chapter 5 deals with the prevention of disability, separately for the developing and for the industrialized countries. In the developing countries, at the first level of prevention, the prime objectives are to promote socioeconomic development and to provide improved health conditions for the 800 million human beings who are living in extreme poverty. Within these general measures, fight against malnutrition and infectious diseases prevention and more

efficient management of accidents and improvement of perinatal care belong to the primary tasks. The second level of prevention implies provision for the necessary drugs against leprosy, tuberculosis, ear infections, epilepsy, psychoses, hypertensive disease, diabetes and trachoma and for the necessary surgical interventions such as treatment of wounds, fractures and of other injuries to the limbs, operations for cataract etc., and as far as possible for rehabilitation. In the industrialized countries prevention has to be directed first of all at traffic accidents and occupational and domestic injuries. Early diagnosis and therapy of chronic organic diseases such as rheumatism, diseases of the heart and circulation, respiratory and mental diseases, genetic defects, etc. are also major fields of prevention.

Chapter 6 reviews the situation of rehabilitation in the industrialized countries. Some questions of organization, provision for specialized manpower, management and supervision are discussed and recommendations are given. Chapter 7 relating to the developing countries is of the same context as the former, but the recommendations are covered by Chapter 8. This is an exhaustive chapter providing a great deal of factual information. Cooperation with WHO and coordinated work of other organizations and agencies are outlined in Chapter 9. Chapter 10 contains general recommendations for the governments of the individual countries.

There is a list of references and an appendix with examples of provisions and measures.

L. Cselkó

Medical certification of cause of death. WHO, Geneva, 1979. pp. 27, Sw. Fr. 2.—

This booklet now in its fourth edition has the aim, to give the physician clear instructions on the international standards he has to comply with on issuing a medical certificate of the cause of death.

The introduction points out the significance of certification and discusses the historical backgrounds of the present recommendations. A separate chapter offers a basis for the identification of the causes of death and recommends a printed form for this purpose. Then the main causes of death are listed in detail. The remaining part of the booklet is a collection of case records. We find here 21 models for death certificates, with one or more different disease as the cause of death in each case. These models are useful as they cover practically the entire international list of causes of death.

As a guide for international standardization the booklet will be useful to the practitioner.

L. Cselkó

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 support from the Olympus Optical Company. 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 biological and clinical 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 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:

INTERNATIONAL UNION AGAINST CANCER

Rue du Conseil-Général 3
1205 Geneva
Switzerland

CANCER RESEARCH CAMPAIGN
INTERNATIONAL FELLOWSHIPS

The International Union Against Cancer, with funds provided by the Cancer Research Campaign (UK), will award fellowships for research on cancer. These are designed to enable investigators to work abroad to gain new experience in clinical or basic research in cancer. These fellowships are also open to investigators in the behavioural or social sciences relevant to cancer.

Fellowships will be granted only to persons on the staff of universities, teaching hospitals, research laboratoires or similar institutions. Applicants must have between two and ten years' post-doctoral experience (Ph. D., M. D., DVM) or equivalent.

A fellowship will not be granted to a person who wishes to perfect his training or who wishes to visit briefly several institutions abroad. The duration of the fellowships ordinarily will be one year but this period may be longer or shorter in special circumstances.

The stipend will be fixed on the basis of £ 9.000 per annum adjusted to the cost of living in the host country.

The fellow will receive a travel allowance towards the cost of a tourist/economy class air fare. A similar allowance will be granted to the spouse who wishes to join a fellow for six months or more.

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

rue du Conseil-Général, 3
1205 Geneva
Switzerland

INTERNATIONAL CANCER RESEARCH TECHNOLOGY TRANSFER PROGRAMME

(ICRETT)

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.

The selection of applicants will be on a continuous basis and the results will be communicated as rapidly as possible.

Additional information and applications forms may be obtained from:

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.

ARERICAN CANCER SOCIETY
ELEANOR ROOSEVELT
INTERNATIONAL CANCER FELLOWSHIPS

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.

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FORMERLY ACTA MEDICA ACADEMIAE SCIENTIARUM HUNGARICAE

Acta Medica Hungarica

VOLUME 40, NUMBERS 2–3, 1983

Akadémiai Kiadó, Budapest
ACTA MED. HUNG. 40(2–3) 73–183 (1983) HU ISSN 0236–5286

ACTA MEDICA HUNGARICA

A QUARTERLY OF THE HUNGARIAN ACADEMY OF SCIENCES

Acta Medica publishes reviews and original papers on clinical and experimental medicine in English.

Acta Medica is published in yearly volumes of four issues by

AKADÉMIAI KIADÓ

Publishing House of the Hungarian Academy of Sciences
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Manuscripts and editorial correspondence should be addressed to the Managing Editor:

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Acta Medica Hungarica 40 (2—3), pp. 73—79 (1983)

Immunology

THE OTHER SIDE OF IMMUNOLOGY: IMMUNOPLASIA TROPHIC (PLASTIC) FUNCTION OF THE IMMUNE SYSTEM*

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(Received December 28, 1982)

Destruction of non-self antigens for the preservation of the unique individual antigenic composition independently of the consequences (protection, allergy, death by anaphylactic shock, autoaggression) and serving the higher law of phylogenetic differentiation, is only one side of immune function. There is an equally important other side, the building, plastic or trophic function (immunoplasia, immunotrophy) which helps to produce this unique antigenicity throughout life, gives the initiating signal to regeneration after cell losses and controls cell proliferation until normal shape and size are regained. Failing or overfunctioning immunoplasia may be the cause of organ atrophy or hyperplasia with appropriate diseases as manifesting consequences. To many of the "idiopathic" organ atrophies and hypofunctions immuno-hypoplasia is a better explanation then the supposed autoimmune aggression. Based partly on the author's own experience the data and arguments are summarized which support and reinforce the theory of immunoplasia.

Keywords: immune physiology, immune pathology, immunoplasia, immunotrophy, morphostasis, phylogenetic differentiation

Immunology started with observations of contagious diseases and even before the era of microbiology vaccinations proved their right (Jenner, 1798). The next important step was the production of antisera with specific antibodies (Behring, 1890) and later the detection of immunoglobulins. From the Latin "immunis" and "immunitas" the term immune, immunity and immunology have been coined, meaning protection against diseases and the science dealing with this protection. A revolutionary breakthrough brought forth the discovery that immunity and hypersensitivity have a common basis, an altered reactivity to a secondary antigenic stimulus acquired from a primary stimulus by the very same antigen: the doctrine of *allergy* (Pirquet, 1906) with its immediate (humoral, antibody-mediated) and delayed (cell-mediated) reaction types. So it has become evident that the immune system is not always beneficial but may be harmful as well.

* Opening lecture of the IV. Symposium Allergologicum et Clinico-Immunologicum. Budapest, 15 September, 1982

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The malefactor role of the immune system has been completed in our days when it became clear that the theory of "horror autotoxicus" is no more tenable and autoimmune aggression explains the pathogenesis of many, hitherto "idiopathic" destructive inflammatory diseases.

For the sake of later considerations it is necessary to stress that allergy, even the lethal anaphylactic shock, is in many respects a physiological reaction of the immune system, which ceases if the system has become defective. Immunopathology thus has two meanings, viz. pathologic phenomena caused by the immune system, i.e. allergy and autoimmune aggression; and diseases of the system itself (defects or diseases such as tumours, etc.).

It is hard to understand why a system aimed at protection should be prepared to threaten or destroy life (lethal shock, destruction of a transplanted organ of vital importance or an embryo) with its allergic reaction. My explanation is that the immune system obeys a higher law of life than the safety of the individual. It acts for the higher law of phylogenetic differentiation, protecting by every means the unique antigenic construction of the individuals even at the expense of their destruction [24].

In medical practice we necessarily differentiate between protective (beneficial) and pathogenic (harmful) immune responses, but after all *desantigenisation*, destruction of the specified macromolecules perceived as antigens is the ultimate aim and task of the immune system, irrespective of the consequences.

This definition however is not complete any more. By and by sufficient disturbing data have emerged to show that antigen destruction (elimination) is only one side of immune function and there is another side as well, an opposite side which completes it to a biologic entity, namely a *trophic or plastic function, controlling* cell proliferations, mitoses, the size of the tissues and the shape of the organs (euplasia, metrostasis).

As far as I know the story, immunoplasia, immune control of cell growth, has begun in 1922 when Carrel [9], the greatest name associated with tissue culture, observed that lymphocytes promote the growth of other cells. Since then many investigations have been carried out to clarify the interactions of lymphocytes with other cells [4, 14, 15, 16, 19, 20, 21, 30, 31].

Altough I use alternatively the greek words trophy (trophe = nutrition) or plasia (plasis = developing, moulding, forming) there is some difference between them. Hyperplasia means larger size due to cell proliferation (i.e. more cells) while hypertrophy means larger size due to the cells growing larger but their number remaining the same. So euplasia and eutrophy are perhaps synonims, but as to the function of the immune system, as we shall see, the expression "plasia" is more adequate.

The most primitive method of physiology to examine what an organ is good for is its excision and to see what happens. There have never been doubts about the function of muscle, bone, eye, the trophic action of nerves, some hormones, etc., but just the opposite is the case with the immune system. It is so dispersed throughout the body that it cannot be excised as a whole

and its isolated total destruction by other means is quite impossible. Only lately have we learned about the functions of the spleen, thymus, bursa Fabricii, lymphnodes, B-T-K cells, lymphokines, interleukins, etc., but our knowledge is yet incomplete and in many respects quite uncertain. The field is rather hazy but it begins to brighten.

The existence of *immunoplasia*, the plastic function of the immune system has to be proven from all sides, namely that in cases of hypoplasia–aplasia a failing, in cases of hyperplasia a hyperfunctioning, organ-specific immunoplasia may be the cause, and that immunoplasia has a lifelong continuous action in maintaining the normal shape and size of the organs and tissues constantly losing cells and is ready to induce and control the restoration of tissues losses, regeneration, and wound healing.

Although we have no means to remove the immune system in order to prove the existence of immuno*hypoplasia*, there are fortunately some natural models. In humans to many "idiopathic" organ atrophies (aplasias) a failing organ-specific immunoplasia seems to be a cause more plausible than an autoimmune aggression, and in animals marasmus develops if their lymphoid tissues are hypoplastic.

There is no doubt that an antibody can destroy a cell by attacking a vitally important antigen of its surface. Yet most of the autoantibodies react with antigens not represented on the cell surface (thyreoglobulin, mitochondria, microsomes, DNA, etc.) and therefore can only form immune complexes with non-immune-specific consequences. It is well known that most of the autoantibodies are not pathogenic and occur even in healthy persons [23]. It follows that in most "organ-specific" autoimmune diseases leading to hypoplasia (atrophy) and hypofunction there is no real proof of an autoimmune aggression. Autoantibodies non-pathogenic in themselves prove only that something is wrong with antibody production [22]. The "shower" of different, directly non-pathogenic, non-cell-surface directed antibodies in SLE, the prototype of autoimmune diseases, show in the same direction, to derailment of immune regulation, but beyond the non-specific immune complex depositions we have no proof of autoimmune aggression as the real background of the disease. We know that in animals severe lymphoplasia of the lymphoid tissues goes in hand with wasting (runt disease, secondary disease). The fact that in sterile surroundings some of these animals survive allows the conclusion that a resistant lymphoid cell population must be responsible for the immunoplasia. The main problem is that for the moment we have no simple means to quantitate the actual immunoplastic capacities. While we have no direct evidence of their failure as the cause of these hypoplasias, at the same time there is no evidence against it.

Let us continue our considerations with the *hyperfunction of immunoplasia*. We have to look after transferable circulating factors. The first impetus

came from the discovery that the long acting thyroid stimulator (LATS) is a gamma globulin, a thyroid stimulating immunoglobulin [1, 2, 18] and so Graves' disease had to be classified into the group of autoimmune diseases. The essence, however, is that an immune stimulation instead of destructive inflammation causes hyperplasia and hyperfunction [12]. If there is one example in human pathology, there should be others too. We ourselves tried to search stimulating immunoglobulins in other hyperplasias and have shown that the blood of peptic ulcer patients contains a stimulating immunoglobulin which is transferable to rats causing in them after a time peptic ulcer mediated by the hyperplasia of the acid secreting parietal cell mass [11, 25]. We have preliminary data [26], and there is also a paper in the literature too [3] showing that a stimulating immunoglobulin may be the promoter of the bilateral adrenal hyperplasia with the clinical consequences of Cushing's syndrome. I think it is wortwhile to follow this line looking for other hyperplasias. Seek and you will find. Since these hyperplasias are mostly organ-specific, occurring at a time only in one organ, immunoplasia too must be organ-specific. This, however, does not exclude the possibility that at the same time several organs or endocrine glands may be involved in immunoplastic disparity, causing for instance multiple endocrinopathy. There are of course many problems with the research of immunohyperplasia, the most important being the lack of suitable animal models.

To prove the existence of the lifelong acting and ever ready normal immunoplasia *(immunoeuplasia)* seemed at first difficult but soon it has become clear that we have suitable animal and human models for its investigation in the processes of *regeneration*.

Probably every kind of regeneration could be an equally good method for diagnosing the effectivity of immunoplasia but until now the greatest bulk of experience has been furnished by partial hepatectomy and unilateral nephrectomy. The question is what compels the cells to proliferate, what is the first stimulus, the initiating signal and later what is the limiting factor when the organ loss has been recovered and the normal size and shape has been regained. I am convinced that only immunoplasia can give the correct answer.

A 2/3 liver loss by hepatectomy sets in motion an immediate burst of astonishingly rapid reparative cell proliferation in the remaining lobes. The growth is precisely regulated and ceases when the deficit has been restored. Not only the rate, degree and shape of the restoration is peculiar but the fact too that function lags far behind the cell proliferation, the compensation of work-load being not the cause but the consequence of the hyperplasia [6].

Another good model for the investigation of immunoplasia is the so-called "compensatory" hypertrophy of the remaining kidney after unilateral nephrectomy. Although this phenomenon has been known since the end

of the last century [28], the connection of its mechanism with the immune system is relatively new. The quick response is very similar to that of an immune reaction [8, 13]. In contrast to the liver some investigators think that this is rather a hypertrophy (hence the name). It is true that no new glomeruli are formed, but the enlargement of the nephrons is established by cell proliferation.

Many details of the immunological and trophic function of lymphocytes have been studied by Kellsall and Crabb [17]. The role of the lymphoid tissue in morphostatis has been suggested by Burwell [8].

Burch and Burwell [7] supposed that the organs release "tissue coding factors" (TCF) into the extracellular fluid and the rate is related to their size and cellularity. These factors pass via the efferent lymphatics into the regional lymph nodes where they act as afferent signals to the controlling lymphoid tissue which sends small effector lymphocytes with mitotic control proteins to the target tissue and so promote cell proliferation. After tissue loss the reduction of the quantity of the factors would be the stimulus and hyperplasia would go on until an equilibrium has been created, the debt has been acquitted. Burch and Burwell presented in detail their theory as a unified biological concept of embryogenesis, cellular differentiation, mitotic and growth control, autoimmunity, carcinogenesis and ageing. They postulated that the primary and intrinsic function of the complex lymphoid system is the control of the growth and size of target tissues throughout the body. They opposed Burnet's clonal selection theory with clonal induction, self recognition having a positive relationship between the specific elements of the lymphatic system and the antigenic determinants of other tissues. In contrast with the previous theory that the immune system carries out purely police actions, they suggested that defence function is a secondary concomitant with the primary regulation of tissue size.

Based on many dispersed data, observations in the literature and our own clinical and experimental experiences, I think that the plastic action of the immune system is neither selected nor induced, but predetermined by a rather fixed germ-line number of organ-specific lymphoid trophocytes, their number being in equilibrium with the number of cells they care for. They recirculate between their homing target and regional lymph nodes regularly for refuelling, the lymph nodes serving like naval bases, or aircraft carrier nurse ships. If the organ-specific trophocytes are in excess, they induce proliferation until the equilibrium has been restored, whereas their insufficiency in number or function leads to hypoplasia or atrophy.

Other regeneration models are available as well for the investigation of the morphostatic function of the immune system, such as wound healing in vivo (especially of standardized skin and mucous membrane wounds), the epidermal growth factor [10] and the erythroid burst forming units [27, 29,

32] in vitro. A basic question is, how the information carried and produced by the specific feeder cells reaches the cells in a proliferating tissue. The investigation of "lymphocyte induced angiogenesis" gives the answer [5, 16].

In a few pages it is impossible to give a whole account of an evolving new science which completes the biology of the immune system. Being at the beginning of exploring the other side of immune function, of immunoplasia, many open questions and doubts remain. Much work awaits the researchers to prove or disprove allegations.

REFERENCES

1. Adams, D. D.: The presence of an abnormal thyroid-stimulating hormone in the serum of some thyrotoxic patients. J. Clin. Endocrinol. and Metab. **18,** 699–712 (1958).
2. Adams, D. D., Purvis, H. D.: Abnormal responses to the assay of thyreotrophin. Proc. Univ. Otago Med. Sch. **34,** 11–17 (1956).
3. Andrada, J. A., Murray, F. T., Andrada, E. C., Ezrin, C.: Cushing's syndrome and autoimmunity. Arch. Path. Lab. Med. **103,** 244–246 (1979).
4. Andrew, W., Andrew, N. V.: Lymphocytes in the normal epidermis of the rat and of man. Anat. Rec. **104,** 217–232 (1949).
5. Auerbach, R., Sidky, Y. A.: Nature of the stimulus leading to lymphocyte-induced angiogenesis. J. Immunol. **123,** 751—754 (1979).
6. Bucher, N. L., Malt, R. A.: Regeneration of Liver and Kidney. Little, Brown-Co., Boston 1971.
7. Burch, P. R. J., Burwell, R. G.: Self and not-self. A clonal induction approach to immunology. Quart. Rev. Biol. **40,** 252—279 (1965).
8. Burwell, R. G.: The role of lymphoid tissue in morphostasis. Lancet **2,** 69–74 (1963).
9. Carrel, A.: Growth promoting function of leukocytes. J. exp. Med. **36,** 385–391 (1922).
10. Das, M.: Epidermal growth factor action as a model for lymphokine function. Fed. Proc. **41,** 2484–2489 (1982).
11. Dobi, S., Petrányi, Gy.: Secretagogue globulins in the blood of duodenal ulcder patients. Acta Med. Acad. Sci. Hung. **30,** 205–210 (1973).
12. Erdei, I., Fazakas, S., Kiss, B., Szegedi, Gy., Petrányi, Gy.: The autoimmune status in Graves' disease. Acta Med. Acad. Sci. Hung. **31,** 173–179 (1974).
13. Fox, M., Wahman, G. E.: Etiology of the compensatory renal response. Observations on the role of the lymphoid system. Invest. Urol. **5,** 521–538 (1968).
14. Goudie, R. B., Macfarlane, P. S., Lindsay, M. K.: Homing of lymphocytes to non-lymphoid tissues. Lancet **1,** 292–293 (1974).
15. Humble, J. G., Jayne, W. H., Pulvertaft, R. J.: Biological interaction between lymphocytes and other cells. Brit. J. Haemat. **2,** 283–294 (1956).
16. Kaminsky, M. J., Nowacyk, M., Skopinska-Rozewsk, E., Kaminska, G., Bem, W.: Human peripheral blood T lymphocyte subpopulation isolated on the basis of their affinity for sheep red-cells differ in angiogenesis-producing capability. Clin. Exp. Immunol. **46,** 327–331 (1982).
17. Kellsall, M. A., Crabb, E. D., cit. Loutit (19).
18. Kriss, J. P., Pleshakov, V., Chien, J. R.: Isolation and identification of the long-acting thyroid stimulator and its relation to hyperthyroidism and circumscribed pretibial nyxoedema. J. Clin. Endocrinol. and Metab. **24,** 1005–1028 (1964).
19. Loutit, J. F.: Immunological and trophic functions of lymphocytes. Lancet. **2,** 1106–1108 (1962).
20. Loutit, J. F.: Lymphocytes. Brit. J. Radiol. **36,** 785–794 (1963).
21. Needham, A. E.: Chapter 7 In: Nowinski, W. W. (ed.) Fundamental Aspects of Normal and Malignant Growth. Amsterdam 1959.
22. Petrányi, Gy.: Naturgeschichte des Lupus Erythematodes und die klinische Zusammenhänge der rheumatischen Erkrankungen. Acta Med. Acad. Sci. Hung. **12,** 85–105 (1958).
23. Petrányi Gy.: Az autoantitestek pathogenetikai szerepe (Pathogenic role of autoantibodies). Orv. Hetil. **106,** 1873—1879 (1965).
24. Petrányi Gy.: Immunfiziológia—immunpatológia (Immune physiology—immune pathology). Orvosképzés Suppl. **45,** 3–12 (1970).

25. Petrányi, Gy.: A tápcsatorna autoimmun betegségei (Autoimmune diseases of the digestive tract). Orv. Hetil. **121,** 127–132 (1980).
26. Petrányi, Gy., Rácz, K.: Stimulating antibody in the blood of a patient with bilateral micronodular hyperplasia, Cushing's syndrome and SLE (unpublished).
27. Porter, O. N., Ogawa, M.: Characterisation of human erythroid burst-promoting activity derived from bone marrow conditioned media. Blood **59,** 1207–1212 (1982).
28. Sacerdotti, C.: Über die kompensatorische Hypertrophie der Niere. Virchow's Arch. Path. Anat. **146,** 267–297 (1896).
29. Torok-Storb, B., Martin, P. J., Hansen, J. A.: Regulation of in vitro erythropoiesis by normal T cells: Evidence for two T cell subsets with opposing function. Blood **58,** 171–174 (1981).
30. Trowell, O. A.: The Lymphocyte. Int. Rev. Cytology. **7,** 235–293 (1958).
31. Weiss, P.; Self-regulation of organ growth by its own products. Science **115,** 487–488 (1952).
32. Zuckerman, K. S.: Human erythroid burst-forming units. Growth in vitro dependent on monocytes but not T lymphocytes. J. Clin. Invest. **67,** 702–709 (1982).

Acta Medica Hungarica 40 (2—3), pp. 81—89 (1983)

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(Received May 31, 1982)

The number of autologous rosette forming cells was studied in patients with SLE. The effect of steroid hormones and of sera derived from SLE patients and normal controls on autologous rosette formation by lymphocytes of SLE patients and of normal controls was examined. The number of T lymphocytes capable of recognizing autologous red cells was found to be significantly lower in the SLE patients than in the group of normal controls. Steroid hormones, whether applied in vivo or in vitro, displayed an inhibitory effect on autologous rosette formation in SLE patients and normal controls alike. The effect in vitro was dose-related, and there was no difference in sensitivity between normal and SLE lymphocytes. Pooled sera of high immunocomplex content obtained from SLE patients were also found to reduce the number of autorosettes. Since the lymphocytes capable of recognizing autologous red cells are known to be post-thymic precursor cells equally subject to differentiation in helper and suppression direction and play an important role in the regulatory mechanism, reduction in their number or disturbances in their function and differentiation may well provide one of the factors accounting for the impairment of immune regulation in SLE.

Keywords: autologous rosette forming T cells, steroid hormones, pooled sera, immune regulation

Introduction

One of the properties of human T lymphocytes is to form rosettes with erythrocytes of various species. Rosette formation may even occur with the subject's own erythrocytes [10, 17, 34].

30 to 40% of human thymocytes and approximately 25% of peripheral lymphocytes of normal subjects are capable of binding autologous erythrocytes [3, 5, 26, 30]. The lymphocytes capable of rosette formation with autologous erythrocytes represent a precursor T cell population in which functionally still non-committed lymphocytes are also represented [25, 26, 27]. These cells are sensitive to steroid hormones in vivo and in vitro, they are resistant to theophylline and they lack an IgG or IgM binding Fc receptor structure. These T cells play an important part in the maintenance of feedback inhibition [21, 25]. Recently it has been shown that a number of these cells has already the capacity of reacting with the monoclonal antibodies

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OKT 4 and OKT 8 [12, 32]. Differentiation of the post-thymic precursor autologous rosette forming cells (ARFC) to effector cells may be induced by thymic hormones and by pretreatment with nonspecific mitogens [2, 30].

The aetiology of SLE is attributed to disturbances in immune regulation. Reduction in the number of the suppressor precursor T cells or an inadequacy of their differentiation, may provide one of the contributing factors.

The present study has been concerned with the autologous rosette-forming capacity of peripheral lymphocytes of SLE patients. Autologous rosette formation by lymphocytes of normal donors and of SLE patients under the effect of sera of normal subjects and of SLE patients was studied. Finally, it was examined to what extent the ability of normal and SLE lymphocytes to recognize autologous erythrocytes is affected by steroid treatment in vitro and in vivo.

Patients and methods

The peripheral lymphocytes of 30 normal control subjects of 36.5 years mean age and of 43 patients with SLE (5 males and 38 females, of 45.6 years mean age) were examined. Diagnosis of SLE was based on the ARA criteria [4]. 20 patients were in an active, 23 in a inactive period at the time of study. A sedimentation rate over 40 mm/h, LE-positivity, impairment of renal function, aggravation of muscle and joint pains, progression of existing skin changes' appearance of serositis were regarded as signs of activity. Nine of the patients with active SLE had histological evidence of lupus nephritis. The patients were given 15 to 30 mg prednisolone daily, the average duration of treatment was 3.5 years. Cytostatic therapy for reducing the dose of the steroid was applied in five cases because of the progressing nephritis.

Drug therapy was withheld during the last 24 hours before the studies.

For the separation of lymphocytes the method of Böyum [1] was used. After the last washing the lymphocytes were taken up in (Ca- and Mg-free) Hank's solution pH 7.4, and the cell count was adjusted to 2×10^7/ml.

The autologous erythrocytes were washed in Hank's solution free from Ca and Mg, and a cell suspension of 3×10^8/ml was prepared.

Autologous rosette test. To a mixture of 0.1 ml lymphocytes (2×10^6) and 0.1 ml autologous erythrocytes (3×10^7), 0.1 ml fetal calf serum (FCS) exhausted with group AB human erythrocytes was added in a test tube. Centrifugation at 200 *g* at room temperature for 5 min was followed by incubation for 24 h at +4 °C. Two min before resuspension the lymphocytes were stained with a drop of 0.2% toluidine blue in Ca- and Mg-free Hank's solution counted. The autologous rosette-forming capacity was expressed in the number of rosettes with at least 3 erythrocytes per 200 lymphocytes.

Effect of steroid treatment on autologous rosette formation in vitro. The lymphocytes of 10 normal controls and 5 SLE patients (2×10^7 per ml) were incubated with prednisolone dissolved in PBS buffer of 2×10^{-3} mmol/l and 1×10^{-2} mmol/l, respectively, at 37 °C for 1 h, then the lymphocytes were incubated with autologous erythrocytes and with FCS exhausted with human group AB erythrocytes and rosetting was examined.

The lymphocytes of two control subjects and of four SLE patients who had received steroids sporadically were studied for steroid effect in vivo. 100 mg prednisolone was administered by the venous route blood was withdrawn prior to, treatment and at 4 and 24 h later, and the rosettes were counted in each sample.

Pretreatment of lymphocytes with the serum of normal and of active SLE patients. To the lymphocytes of ten normal controls and of ten SLE patients, separated on Ficoll, inactivated pooled sera of five normal controls and inactivated pooled sera of five patients with active SLE, were added.

The normal and the SLE lymphocytes (2×10^7/ml) were incubated with 250 μl inactivated normal and SLE serum, respectively, at 37 °C for 30 min. After three washings the test for autologous rosettes was performed as described above. The number of autologous rosettes thus obtained was compared with the rosette count of the untreated controls and SLE patients. For statistical analysis, Student's *t* test was used.

Results

The proportion of ARFC in the lymphocyte population of the 43 SLE patients was considerably lower than in the normal controls (Table I). The fall in the number of ARFC of the active SLE patients was still more marked in the group of lupus nephritis (active SLE 10.8% ±5.7; lupus nephritis 7.7 ±2.1% $P < 0.001$).

Under the effect of steroid hormones in vitro the number of ARFC of both normal controls and SLE patients was found to decline. Elevation of the

Table I

Number of autologous rosettes in normal controls and SLE patients

		Mean number of autologous rosettes, per cent mean ± S.D.	
Normal controls	n = 30	20.4 ± 3.1	$P < 0.001$
Patients with SLE	n = 43	11.3 ± 4.9	
Patients with active SLE	n = 20	10.8 ± 5.7	N.S.
Patients with inactive SLE	n = 23	11.7 ± 4.0	

Table II

Effect of glucocorticoids in vitro on autologous rosette formation, mean ± S.D.

		Initial value	Prednisolone	
			2×10^{-3} mmol/l	1×10^{-3} mmol/l
Controls	n = 10	23.0 ± 2.7	16.0 ± 1.44**	8.8 ± 1.1***
SLE	n = 5	9.8 ± 1.4	4.8 ± 1.1**	3.8 ± 1.4***

Values obtained after incubation of the lymphocytes with prednisolone at 37 °C for 1 h
** = $P < 0.01$
*** = $P < 0.001$

Table III

Effect of glucocorticoids in vivo on autologous rosette formation, mean ± S.D.

		Initial value	At 4 h	At 24 h
Controls	n = 2	22.0 ± 1.0	14.0 ± 1.0	19.5 ± 1.5
SLE	n = 4	9.4 ± 1.1	5.0 ± 0.7	8.7 ± 0.8

Number of autologous rosettes in blood withdrawn at 4 and 24 h after i.v. administration of 100 mg prednisolone

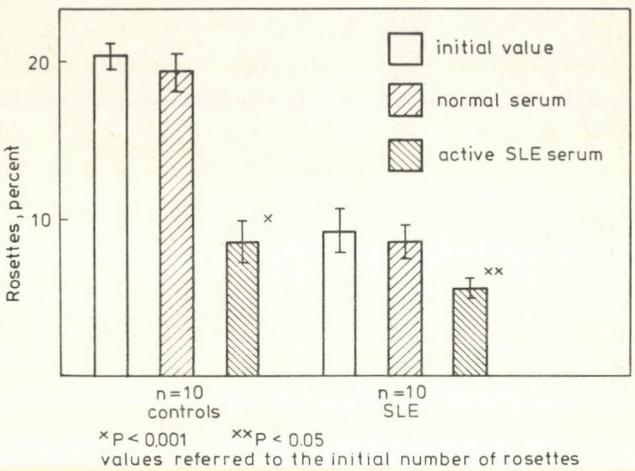

Fig. 1. Effect of sera on antologous rosette forming cells (ARFC)

steroid concentration resulted in a still more marked fall in the number of normal and SLE ARFC alike (Table II).

At 4 h after administration of 100 mg prednisolone a reduction in the autologous erythrocyte binding capacity of the lymphocytes of the controls and the SLE patients was found. By the 24th h the initial values were nearly restored. (Controls: initial value 22.0 $\pm$ 1.0%. At 4 h, 14.0 $\pm$ 1.0%; at 24 h, 19.5 $\pm$ 1.5%. SLE: initial value 9.4 $\pm$ 1.1%. At 4 h, 5.0 $\pm$ 0.7%, at 24 h, 8.7 $\pm$ 0.8%.)

The autologous rosette-forming capacity of normal lymphocytes was unaffected by normal sera. Pretreatment of both normal and SLE lymphocytes with active SLE serum resulted, however, in a significant reduction in the number of ARFC (Fig. 1). The effect of normal serum on normal lymphocytes caused a change from 20.2 $\pm$ 2.4 to 19.6 $\pm$ 3.0%; on SLE lymphocytes from 9.3 $\pm$ 3.5 to 8.5 $\pm$ 2.9%. Under the effect of serum from patients with active SLE: the change in the controls was from 20.2 $\pm$ 2.4 to 8.5 $\pm$ 3.0% ($P < 0.001$) and in SLE from 9.3 $\pm$ 3.5 to 5.8 $\pm$ 1.8% ($P < 0.05$).

Discussion

The rosette forming lymphocytes in the autologous system, the mechanism of rosette formation and the factors likely to influence it have been extensively studied in laboratory animals, especially in mice [15, 21, 35, 36].

The T lymphocytes capable of collecting autologous erythrocytes have only received attention in the last two years, when several studies have been published on their functions and surface marker properties.

According to present knowledge, the ARFC are precursor cells migrating from the thymus. Part of these cells binds peanut agglutinin [22] as a sign that this subpopulation has not yet achieved full maturation. Another part already binds anti-monoclonal OKT 4 and OKT 8 T cell antisera [12, 32]. These are lymphocytes incapable of phagocytosis, carrying no surface Ig, giving a positive reaction with alpha-naphthyl acetate esterase (ANAE), reacting with suboptimal doses of concanavalin acetate (Con-A) but showing no blast transformation. Thus they form a lymphocyte T precursor population resistant to phytohaemagglutinin (PHA), pokeweed mitogen (PWM) and theophylline.

The ARFC exhibit a high sensitivity to steroid hormones in vitro as well as in vivo. The present study has confirmed that steroids applied in vivo or in vitro reduce the number of ARFC in normal subjects and SLE patients alike. The in vitro effect is dose-related and there are no differences in sensitivity between the lymphocytes of SLE patients and normal subjects. The lymphopenic effect of steroids has been shown by Fauci et al. [7]. According to studies in vivo of these authors, this steroid-induced lymphopenia, which primarily affects the T-population, reaches its peak 4 to 6 h after steroid administration and generally ceases by the 24th h. The primary cause of the reduction in lymphocyte count may be sought in an outpouring from the blood stream and in a redistribution mainly into the bone marrow, rather than in a cell destruction [37].

On the other hand, immunologically immature precursor cells and suppressor cells of the thymic cortex are particularly sensitive to steroids in the early phase of maturation, and exposure to steroids of activated T lymphocytes results in their destruction [8]. The mechanism of steroid-induced cytolysis in vitro is still uncertain. What we know is that free fatty acids accumulate, uptake of glucose and nucleotides decreases and ATP production is inhibited by steroid hormones. It is assumed that the steroid receptor complexes formed in the cytoplasm gain access to the nucleus and initiate the production of a specific mRNA, a protein which is thought to play a part in cell destruction.

There is mounting evidence that the lymphocytes taking part in ARFC are actually precursor cells the differentiation of which under the effect of various mitogens may take the direction to T_G or to T_M. It has been shown that the ARFC play an essential part in the regulation of immune processes [20, 26[.

T cytopenia, in other words, an abnormal shift of the helper/suppressor balance, in SLE results in the first place from a reduc tion in the number of cells considered to be of the suppressor type, and to some degree from a functional damage to the T lymphocytes [6, 33, 38]. We have no satisfactory explanation for the complex pathomechanism accounting for the shift in the helper/suppressor proportion.

There are several data to show that the number of ARFC, regarded as precursor lymphocytes, is significantly lower in SLE patients than in normal subjects. Heijnen et al. gave a figure of 4% for autologous rosettes in patients with active SLE [12]. According to Palacios et al., autologous erythrocyte-binding lymphocytes are T cells of regulatory function, the absence of which leads to a B cell hyperfunction by the depression of feedback inhibition [26]. It has been confirmed by Heijnen et al. in vitro that differentiation of the ARFC from suppressor precursor cells to effector suppressor cells takes place only under the effect of antigen-pretreated T_M cells [12]. It is this T_M cell induced suppressor activity which is deficient in SLE because of the absence or inadequate number of autologous rosette forming T lymphocytes.

It has been shown by means of T-cell-specific antisera that the ARFC represent a heterogeneous population. According to the findings of Heijnen, in the purified fraction 51% give a positive reaction with monoclonal antiserum OKT 4, and 34% with OKT 8. In our preliminary studies conducted in collaboration with Benczur, positivity with OKT 4 and OKT 8 was found in 60 and 20%, respectively. It seems that in normal subjects not only the OKT 8, but also the OKT 4 positive ARFC are capable of differentiation to cells of suppressor function, and that in SLE this very process of differentiation is at fault. This is in agreement with the observations of Kammer et al. [16]: while the theophylline resistant OKT 4 lymphocytes of normal individuals assumed OKT 8 surface marker characters in response to adenosine stimulation in vitro, in SLE lymphocytes adenosine failed to induce maturation in the suppressor direction [16].

Recent evidence indicates that the thymic hormones applied in vitro affect primarily the ARFC, and to a lesser degree the T_G cells, while leaving DNA synthesis by the T_M cells and B lymphocytes unaffected [27]. According to earlier observations by Horovitz et al., α_7-thymosine applied in vitro in nanogram quantities restores the deficient suppressor function in SLE [14]. In autologous mixed lymphocyte cultures the ARFC are more responsive than other T cells. It was, on the other hand, possible to improve the deficient responsiveness of ARFC by application in vitro of thymic hormones [28].

Antilymphocyte antibodies, demonstrable in large numbers in the blood of SLE patients, are autoantibodies affecting the functions of lymphocytes. They are responsible for the deficient responsiveness of lymphocytes to non-specific mitogens and may also play a part in the loss of T cells of suppressor type [11, 18, 23, 33, 39, 40]. The cytotoxic IgM antibodies are destructive to the precursors of the suppressor T cells as well. According to Moretta et al. [24] and Giacco et al. [9], the antibodies in the serum of patients with active SLE are inhibitory to spontaneous rosette formation, as also to the functional activity of the Fc-receptors of T_G cells. It has been shown by Láng et al. that sera of high immunocomplex positivity from patients with active SLE are

inhibitory to the antibody-dependent cytotoxicity of normal human lymphocytes [20]. The immunocomplex in the SLE sera not only affect the function of the K cells but also that of the natural killer (NK) cells [11, 13].

Published data relating to the NK activity of ARFC are inconsistent. While according to Palacios et al. [29] the ARFC are precursors of the NK cells, the results of Rucheton et al. are at variance with any NK activity of this cell population [32]. According to our results the antibodies in the sera of patients with active SLE are capable of inhibiting autologous rosette formation by the precursor T cells. We lack any accurate knowledge about this inhibitory mechanism. The most likely explanation seems to be that the antibodies alter the surface membrane structure of the T cells, thus causing a loss of their capacity to recognize autologous erythrocytes. This is assumed to affect autologous erythrocytes. This is assumed to affect the capacity of differentiation of this cell population and to result in further T cell damage.

The disturbed function of T cells in SLE is attributed to multiple factors. It seems justified to assume a genetically determined functional T cell deficiency resulting in an arrest of maturation of the T cells being under way of differentiation in the suppressor direction. Further qualitative and quantitative alterations of the T cells result from the presence of autoantibodies characteristic of SLE.

From the results of the present study it emerges that among the very heterogeneous T cells there exists a lymphocyte population capable of recognizing autologous antigenic structures. This population which reacts sensitively to autoantibodies of SLE sera is quantitatively affected in SLE and is assumed to play a part in the abnormal shift of the helper–suppressor balance associated with this disease.

REFERENCES

1. Böyum, A.: Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Lab. Invest. **21,** 77–89 (1968).
2. Bach, J. F., Bach, M. A., Charreire, J., Dardenne, M., Plau, J. M.: The mode of action of thymic hormones. Ann. N. Y. Acad. Sci. **332,** 23–32 (1979).
3. Caraux, J., Thierry, C., Esteve, C., Flores, G., Lodise, R., Serrou, B.: Human autologous rosettes I. Mechanism of binding of autologous erythrocytes by T cells. Cell. Immunol. **45,** 36–48 (1979).
4. Cohen, A. S., Reynolds, W. E., Franklin, E. C.: Preliminary criteria for the classification of systemic lupus erythematosus. Bull. Rheum. Dis. **21,** 643–647 (1971).
5. Cupissol, D., Rey, A., Favier, F., Serrou, B.: In vitro and in vivo effect of a sugar derivative of nitrosourea (RFCNU) on immune response in cancer patient. Nitrosoureas in Cancer Treatment, INSERM Symposium 19., 1981.
6. Delfraissy, J. F., Segond, P., Wallon, C., Massias, P., Dormont, J.: Depressed primary in vitro antibody response in untreated systemic lupus erythematosus. J. Clin. Invest. **66,** 141–148 (1980).
7. Fauci, A. S., Murakami, T., Brandon, D. D., Loriaux, D. L., Lipsett, M. B.: Mechanism of corticosteroid action on lymphocyte subpopulation. Cell. Immunol. **49,** 43–50 (1980).
8. Galili, N., Galili, U., Klein, E., Rosenthal, L., Nordenskjöld, B.: Human T lymphocytes become glucocorticoid-sensitive upon immune activation. Cell. Immunol. **50,** 440–444 (1980).

9. Giacco, G. S., Mathieu, A., Loy, M., Locci, F., Cengiarotti, L., Leone, A. L.: E rosette inhibition in systemic lupus erythematosus. Arthr. and Rheum. **23,** 1406–1407 (1980).
10. Gluckman, J. C., Montambault, P.: Spontaneous autorosette-forming cells in man. A marker for a subset population of T lymphocytes? Clin. Exp. Immunol. **22,** 302–310 (1975).
11. Goto, M., Tanimoto, K., Horiushi, Y.: Natural cell mediated cytotoxicity in systemic lupus erythematosus. Arthr. and Rheum. **23,** 1274–1281 (1980).
12. Heijnen, C. J., Pot, K. H., Kater, L., Kluin-Nelemans, H. C., Uytdehaag, F., Ballieux, R. E.: Functional analysis of the defective T cell regulation of the antigen-specific PFC response in SLE patients: Differentiation of suppressor precursor cells to suppressor effector cells. Clin. Exp. Immunol. **47,** 359–367 (1982).
13. Hoffman, J.; Natural killer function in systemic lupus erythematosus. Arthr. and Rheum. **23,** 30–35 (1980).
14. Horowitz, S., Borchering, W., Moorthy, A. V., Chesney, R., Wisserman, H. S., Hong, R.: Induction of suppressor T cells in systemic lupus erythematosus by thymosine and cultured thymic epithelium. Science **197,** 999–1001 (1977).
15. Hsu, K. L., Ghanta, V. K., Duncan, L. A., Hunt, C. E., Hiramoto, R. N.: Characterisation of autorosette inhibitory fraction in mouse serum. J. Immunol. **3,** 1298–1301 (1981).
16. Kammer, G. M., Birch, R. E., Polmar, S. H.: Defective adenosine-induced suppressor T-lymphocyte generation in systemic lupus erythematosus. Case Western Reserve University, Cleveland (1981).
17. Kaplan, J.: Human T lymphocytes form rosettes with autologous and allogenic human red blood cells. Clin. Immunol. Immunopath. **3,** 471–477 (1975).
18. Kaufman, D. B., Bostwick, E.: Defective suppressor T cell activity in systemic lupus erythematosus. Clin. Immunol. Immunopath. **13,** 9–18 (1979).
19. Láng, I., Fekete, B., Pálóczi, K., Sonkoly, I., Szegedi, Gy., Török, K., Gergely, P., Petrányi, Gy.: Antibody-dependent cellular cytotoxicity in systemic lupus erythematosus. Acta Med. Acad. Sci. Hung. **34,** 259–265 (1977).
20. Láng, I., Török, K., Fekete, B., Gergely, P., Petrányi, Gy.: In vitro effect of immune complex positive human sera on antibody-dependent cellular cytotoxicity. Acta Med. Acad. Sci. Hung. **34,** 117–121 (1977).
21. Limatibul, S., Shore, A., Dosch, H. M., Delfand, E. W.: Theophylline modulation of E rosette formation: an indicator of T cell maturation. Clin. Exp. Immunol. **33,** 503–513 (1978).
22. London, J., Berrih, S., Bach, J. F.: Peanut agglutinin I. A new tool for studying T lymphocyte subpopulations. Immunology **121,** 438–443 (1978).
23. Malave, I., Papa, R., Layrisse, Z.: Lymphocytotoxic antibodies in SLE patients and their relatives. Arthr. and Rheum. **19,** 700–703 (1976).
24. Moretta, A., Mingari, M. C., Santoli, D., Perlman, P., Moretta, L.: Human T lymphocyte alteration in systemic lupus erythematosus. Scand. J. Immunol. **10,** 223–228 (1979).
25. Palacios, R., Alarcon-Segovia, D.: Human post-thymic precursor cells in health and disease. III. Role of the autologous rosette forming T cells in the generation of spontaneous killer cells in the autologous mixed lymphocyte reaction. Scand. J. Immunol. **13,** 499–502 (1981).
26. Palacios, R., Alarcon-Segovia, D., Llorente, L., Ruiz-Arguelles, A., Diaz-Jouanen, E.: Human post-thymic precursor cells in health and disease. I. Characterization of the autologous rosette-forming T cells as post-thymic precursors. Immunology **42,** 127–135 (1981).
27. Palacios, R., Alarcon-Segovia, D., Ruiz-Arguelles, A., Llorente, L.: Human post-thymic precursor cells in health and disease. V. Effect of serum thymic factor on the cell cycle of post thymic precursor cells on their generation of T gamma and T_{μ} cells. Immunol. Letters **4,** 35–38 (1982).
28. Palacios, R., Ruiz-Arguelles, A., Alarcon-Segovia, D.: Human post-thymic precursor cells in health and disease. IX. Immunregulatory T cell circuits in peripheral blood of patients with rheumatoid arthritis. Clin. Exp. Immunol. **43,** 473–477 (1981).
29. Palacios, R., Llorente, L., Alarcon-Segovia, D., Arguelles, A. R., Jouanen, E.: Autologous rosette-forming T cells as the responding cells in human autologous mixed lymphocyte reaction. J. Clin. Invest. **65,** 1527—1530 (1980).
30. Palacios, R.: Role of the autologous rosette-forming T cells in the concanavalin-A-induced suppressor cell function. Cell. Immunol. **61,** 273–279 (1981).
31. Primi, D., Lewis, G. K., Triglia, R., Goodman, J. W.: Rosette formation between murine lymphocytes and erythrocytes. A new locus in the H-2 region. J. Exp. Med. **149,** 1349–1354 (1979).

32. Rucheton, M., Caraux, J., Rey, A., Thierry, C., Esteve, C., Valles, H., Dufer, J., Desplaces, A., Zagury, D., Serrou, B.: Human autologous rosettes. Cell. Immunol. **64,** 312–323 (1981).
33. Sagawa, A., Abdou, N. I.: Suppressor cell dysfunction in systemic lupus erythematosus. J. Clin. Invest. **62,** 789–796 (1978).
34. Sandilans, G., Gray, K., Cooney, A., Browning, J. D., Anderson, J. R.: Autorosette formation by human thymocytes and lymphocytes. Lancet **1,** 27–28 (1974).
35. Sia, D. Y., Parish, C. R.: Anti self receptors IV. Immunogenetics **12,** 587–599 (1981).
36. Scheffel, J. W., Kim, Y. B.: Characterization of autologous erythrocyte rosette forming cells in Minesota miniature swine. Cell. Immunol. **57,** 175–182 (1981).
37. Szegedi, Gy.: A kortikoszteroidok hatása a celluláris immunfunkciókra. Pneumol. Hung. **32,** 111–117 (1979).
38. Szegedi, Gy., Kávai, M., Horváth, S., Sonkoly, I., Szabolcsi, M., Szabó, G.: Histamine-binding receptors and Fc receptors of T lymphocytes in SLE. Ann. Immunol. Hung. **19,** 137–141 (1979).
39. Twomey, J. J., Laughter, A. H., Steinberg, A. D.: A serum inhibitor of immune regulation in patients with systemic lupus erythematosus. J. Clin. Invest. **61,** 712–714 (1978).
40. Wright, J. K., Hughes, P., Gelsthorpe, K., Ward, M., Rowell, N. R.: Antibody dependent and phytohaemagglutinin induced lymphocyte cytotoxicity in systemic lupus erythematosus. Ann. Rheum. Dis. **40,** 11–17 (1981).

Acta Medica Hungarica 40 (2—3), pp. 91—97 (1983)

Endocrinology

THYROID ANTIBODIES IN GRAVES' DISEASE. AN IMMUNOFLUORESCENCE STUDY

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(Received November 22, 1982)

The sera of fifty patients suffering from Graves' disease were analysed by indirect immunofluorescence. Normal human thyroid tissue was used as antigen. Antibodies against four different thyroid antigens were detected i.e. follicular cell microsoma, thyroglobulin, follicular basement membrane and follicular cell nuclei. Two types of antibodies binding to the basement membrane were described. Antibodies against the first and second colloid antigens could also be detected. The results suggest that by indirect immunofluorescence four thyroid antibodies, more than known in the literature, are detectable.

Keywords: Graves' disease, indirect immunofluorescence

Introduction

Thyroid stimulating antibodies distinct from thyrotropin (TSH) were first observed more than twenty years ago and Graves' disease itself is believed to have an autoimmune basis [9, 10, 13]. Five main antigen–antibody systems have been identified, involving different constituents of the thyroid gland: thyroglobulin, microsomal antigen, second antigen of colloid, cell surface antigen and antigen related to the TSH receptor [4]. Furthermore, antibodies reacting with thyroxine and triiodothyronine have also been detected in some cases of Hashimoto's thyroiditis, Graves' disease and idiopathic myxoedema [6, 14].

Antibodies directed against thyroid structures can be detected by different means; among these immunofluorescence seems to be the most sensitive method [3, 11]. As summarized recently by Pinchera et al. [11] the only antibody which can be detected in unfixed thyroid sections by immunofluorescence, is the anti-microsomal one. Antibodies binding to the cell surface antigen are detectable by immunofluorescence on viable cells. Antibodies against thyroglobulin and second colloid antigen could be detected by this method only on fixed sections [11].

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Up to now there are few data in the literature regarding the immunofluorescent aspects of Graves' disease [1, 4, 15]. The aim of the recent work was to study the detectability of antibodies in the sera of Graves' patients by applying indirect immunofluorescence.

Materials and methods

Diagnosis of Graves' disease was based on triiodothyronine uptake, triiodothyronine RIA, thyroxine RIA, TSH RIA, ^{131}I scintiscan and the triiodothyronine suppression test.

Normal human thyroid tissue was used as antigen. Normal thyroid tissue obtained from patients undergoing surgery for thyroid cyst or neoplasm was immediately frozen in liquid nitrogen. Five micron thick sections were cut at —20 °C.

Sera of 50 untreated patients suffering from Graves' disease were diluted 1 : 10, 1 : 20 and 1 : 40. Cases of Hashimoto's thyroiditis and toxic adenoma were excluded.

Indirect immunofluorescence was carried out according to Roitt and Doniach [12]. The second incubation was made with FITC labelled anti human IgG (Hyland).

Localization of fluorescence, (microsomal, colloidal, nuclear, basement membrane) its features (finely granular, roughly granular, linear, confluent, homogeneous, patchy) and intensity were evaluated in every case.

As controls, cryostat sections from the same surgical material were incubated with FITC labelled antihuman IgG. If any kind of fluorescence was detected, the material was not processed for indirect immunofluorescence. Parallel to the indirect fluorescence with Graves' patients' sera, the same procedure was carried out with control sera from patients with ischaemic heart disease with normal thyroid function. If control sera proved to show fluorescent positivity, the material was rejected.

Results

Antibodies against thyroid structures were detected in every case. Most frequently, in 29 out of 50 cases, anti-microsomal antibodies were seen. The fluorescent picture was characterized by a finely granular positivity in the cytoplasm of follicular cells (Fig. 1). There was no antibody deposition in other thyroid structures or in the nuclei of follicular cells.

Antibodies binding to thyroglobulin were detected in 15 cases. In the majority, 13 out of 15 antibodies against the second colloid were identified, but in one case antibodies against the first and second colloid antigen were also observed (Fig. 2).

Antibodies binding solely to the first antigen of colloid appeared in a single case (Fig. 3).

The fluorescent pattern of the second colloid antigen–antibody reaction was homogeneous, sometimes patchy filling out completely the follicular lumina (Fig. 2). The first colloid antigen had different characteristics, a roughly granular, partly patchy fluorescence, mainly in the central area of the follículus (Fig. 3). At other sites there was no fluorescence in these cases.

Antibody binding to the basement membrane of the folliculi was detected in five cases. Two types of fluorescence could be identified: a finely granular, in some places linear, mostly confluent one in four cases (Fig. 4), and a roughly

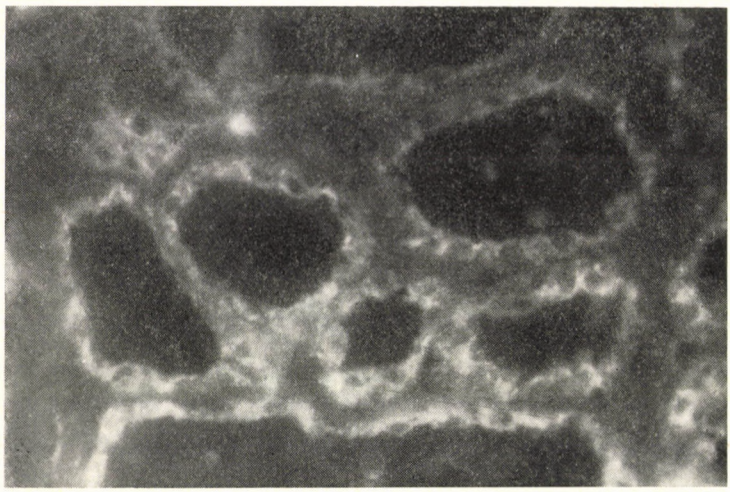

Fig. 1. Anti-microsomal antibody deposition in the cytoplasm of follicular cells. Original magnification, ×400

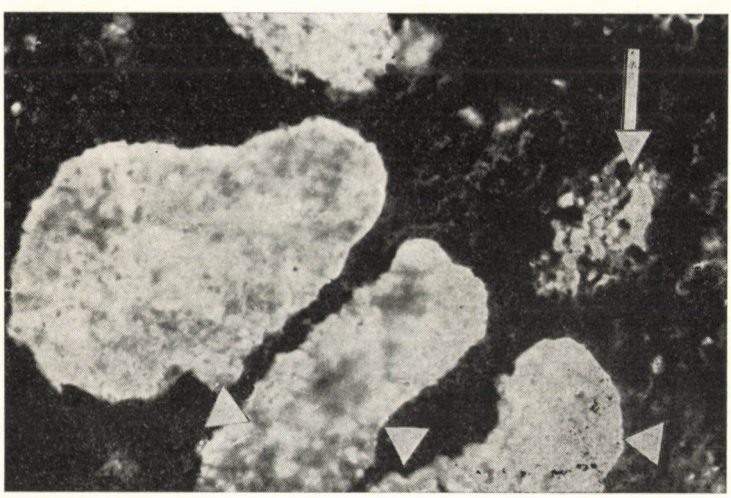

Fig. 2. Antibodies directed against the first (arrow) and second (arrowhead) colloid antigen. Original magnification, ×160

granular, partly linear but not confluent one in one case (Fig. 5). Antibody localized at other sites was not detected in these cases.

Antibody deposition in the nuclei of the follicular cells was seen in a single case. Its pattern was characteristic. The fluorescence was located exclusively in the nuclei of the follicular cells; other structures including the cytoplasm of follicular cells, proved to be negative (Fig. 6). Strangely enough, the dilution rate of the sera had no effect on the intensity of fluorescence. Except three sera which had anti-microsomal and one having anti-basement

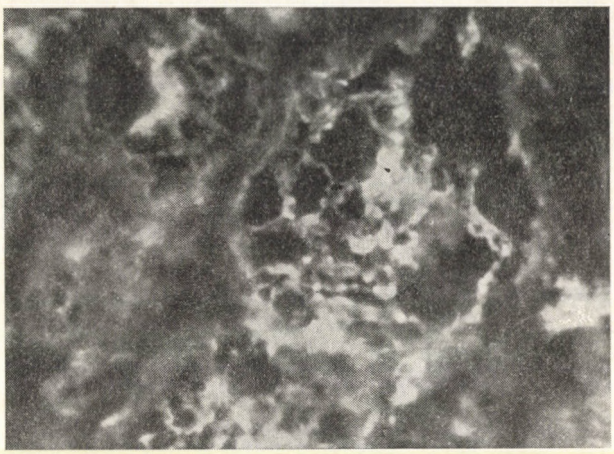

Fig. 3. Antibody directed against the first colloid antigen. Original magnification, ×160

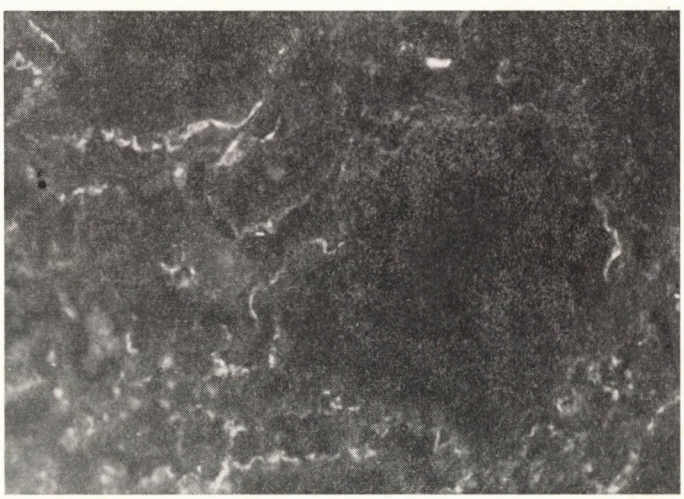

Fig. 4. Antibody deposition in the follicular basement membrane. Finely granular at sites linear, mostly confluent pattern. Original magnification, ×160

membrane antibodies, the intensity did not diminish on further dilution. Even in these cases, only a slight diminution was observed and the positivity remained unambiguous.

Discussion

Indirect immunofluorescence is a sensitive and reliable method for detecting antibodies [4, 7, 16].

In Graves' disease, stimulating antibodies might play a pathogenetic role. According to the data of Doniach [4], five main antigen–antibody systems

are operating in the thyroid gland involving the thryoglobulin, microsoma, colloid, cell surface and TSH receptors. If these systems are really active in the thyroid gland, there is no theoretical hindrance to detect them by indirect immunofluorescence.

The purpose of our work was to describe the different immunofluorescent pictures of the thyroid, using sera of untreated Graves' patients. Immunofluorescent positivity was observed in all of the 50 cases. Microsomal antibodies were the most frequent. Antibodies against the second colloid antigen were less frequent. In 5 out of the 50 investigated sera, antibodies binding to the basement membrane of thyroid folliculi were detected. Two types of deposition could be separated morphologically.

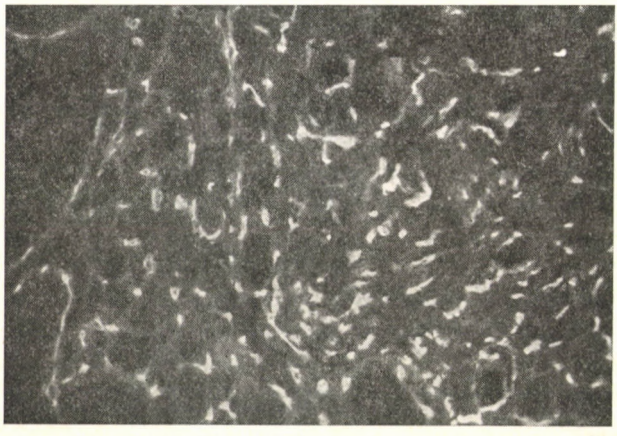

Fig. 5. Antibody deposition in the follicular basement membrane. Roughly granular, partly linear but not confluent pattern. Original magnification, ×160

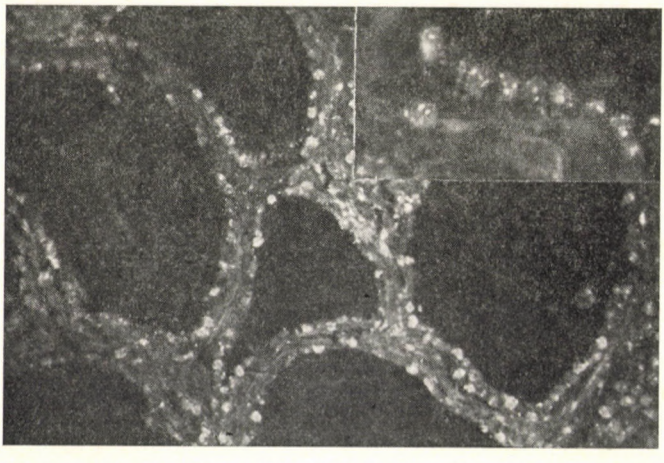

Fig. 6. Antibody deposition in the nuclei of follicular cells. Original magnification, ×160. Inset, ×400

Antibodies binding to the first colloid antigen were seen in a single case and antibodies against both colloid antigens were identifiable in another case. Also, in one case only, antibodies deposited in the nuclei of the follicular cells were detected.

It is difficult to explain our results. It is well known that anti-microsomal and anti-colloidal antibodies are involved in the pathogenesis of Graves' disease [3, 4, 8, 10, 12, 15]. Anti-microsomal and anti-colloidal antibodies were observed in the majority of patients. Taking into consideration the above data the results appear logical.

We have found no data in the literature regarding the detectability of antibodies directed against the follicular basement membrane and the nuclei of follicular cells by applying indirect immunofluorescence. It seems possible that in cases of basement membrane-antibody deposition, thyroid stimulating antibodies which exert their effect on the follicular cell membrane, had been detected. The results of Beall et al. [2] and those of Fagraeus et al. [5] are against this possibility. Khoury et al. [8] observed by indirect immunofluorescence microsomal antigen on the surface of human thyroid cells in culture. It does not seem likely that anti-microsomal antibody binding to the surface of follicular cells was detected in our cases. Namely, anti-microsomal antibodies having a characteristic morphological pattern were identified in the follicular cells.

We could not find any data on the presence and detectability by immunofluorescence of antibodies directed against the follicular cell nuclei in Graves' disease. In this case, too, all the clinical and laboratory findings were indicative of Graves' disease and the possibility of any kind of systemic disorder could be excluded.

Our results thus suggest that by indirect immunofluorescence at least four types of antibody are detectable in the sera of Graves' patients. To explain the clinical and pathogenetical role of these antibodies will be the task of further immunological and clinical investigations.

Acknowledgements

The authors are indebted to Dr. G. Csáky and Dr. G. Lukács for the surgical material.

REFERENCES

1. Akert, F. G., Grob, P. J.: Diagnostische Bedeutung der Schilddrüsenantikörper. Schweiz. Med. Wschr. **107,** 1119–1123 (1977).
2. Beall, G. N., Solomon, D. H., Doniach, D., Roitt, I. M.: Can LATS be detected by immunofluorescence? J. Clin. Endocr. Metab. **29,** 1114–1118 (1969).
3. Bird, T., Stephenson, J.: Evaluation of a tanned red cell technique for thyroid microsomal antibodies. J. Clin. Path. **26,** 623–630 (1973).

4. Doniach, D.: Humoral and genetic aspects of thyroid autoimmunity. Clin. Endocr. Metab. **4,** 267–271 (1975).
5. Fagraeus, A., Jonsson, J., El Kabir, D. J.: What is the antibody specificity of LATS? J. Clin. Endocr. Metab. **31,** 445–447 (1970).
6. Ginsberg, J., Segal, D., Ehrlich, R. M., Walfish, P. G.: Inappropriate triiodothyronine (T_3) and thyroxine (T_4) radioimmunoassay levels secondary to circulating thyroid hormone antibodies. Clin. Endocr. (Oxford) **8,** 133–140 (1978).
7. Hawkins, B. R., Dawkins, R. L., Burger, H. G., Mackay, I. R., Cheach, D. S., Whittingham, S., Patel, Y., Welborn, T. A.: Diagnostic significance of thyroid microsomal antibodies in randomly selected population. Lancet **1,** 1057–1061 (1980).
8. Khoury, E. L., Hammond, L., Bottazzo, G. F., Doniach, D.: Presence of the organ specific "microsomal" autoantigen on the surface of human thyroid cells in culture: its involvement in complement mediated cytotoxicity. Clin. Exp. Immun. **45,** 316–328 (1981).
9. Kriss, J. P., Pleshakov, V., Rosenblum, A. L., Holderness, M., Sharp, G., Utiger, R.: Studies on the pathogenesis of ophthalmopathy of Graves' disease. J. Clin. Endocr. Metab. **27,** 582–586 (1967).
10. MacKenzie, J. M.: Humoral factors in the pathogenesis of Graves' disease. Physiol. Rev. **48,** 252–257 (1968).
11. Pinchera, A., Fenzi, G. F., Bartalena, L., Chiovato, L., Marcocci, C., Pacini, F.: Thyroid antigens involved in autoimmune thyroid disorders. In: Klein, E., Horster, F. A. (eds): Autoimmunity in Thyroid Disease. F. K. Schattauer, Stuttgart–New York 1979.
12. Roitt, I. M., Doniach, D.: Immunofluorescent tests for the detection of autoantibodies. In: WHO Manual of Autoimmune Serology. Geneva 1969.
13. Solomon, D. H., Beall, G. N.: Thyroid stimulating activity in the serum of immunized rabbits. II. Nature of thyroid stimulating material. J. Clin. Endocr. Metab. **28,** 1496–1502 (1968).
14. Staeheli, V., Valloton, M. B., Burger, A.: Detection of human antithyroxine and anti-triiodothyronine antibodies in different thyroid conditions. J. Clin. Endocr. Metab. **41,** 669–675 (1975).
15. Storch, W.: Immunfluoreszenzfibel. G. Fischer Verlag, Jena 1979.
16 Tunbridge, W. M. G.: The epidemiology of autoimmune thyroid disease. In: James. V. H. T. (ed.): Endocrinology. Vol. II. Excerpta Medica, Amsterdam–Oxford 1977.

Acta Medica Hungarica 40 (2—3), pp. 99—105 (1983)

SERUM CALCITONIN IN HYPERTHYROIDISM

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(Received July 21, 1982)

The mineral content of the radius was found to be lower in thyrotoxic than in euthyroid women, particularly in the case of elevated serum triiodothyronine levels. On examining the hormones influencing bone metabolism, the basal serum calcitonin level of thyrotoxic patients was identical with that of the controls. The Ca and calcitonin responses to i.v. Ca loading (3.64 mg $CaCl_2$/kg/3 min) were identical in the euthyroid and hyperthyroid subjects.

The results suggest that the calcitonin reserves in Graves' disease are normal and that the bone abnormalities typical of this disease are unrelated to any change in calcitonin secretion.

The serum calcitonin levels of patients with subacute thyroiditis were identical with those of the controls. An acute fall in the serum calcitonin level occurred in hyperthyroidism, after radioiodine therapy a finding which might be connected with the high radiosensitivity of the parafollicular cells and with a consecutive impairment of calcitonin production.

Keywords: Graves' disease, subacute thyroiditis, calcitonin, Ca loading test, bone mineral content

Introduction

Graves' disease is associated with an increased rate of bone metabolism and with a hyperfunction of osteoblasts and osteoclasts. The bone metabolism is thus shifted towards the resorption processes and results finally in thyrotoxic osteodystrophy [29].

The primary cause of thyrotoxic osteodystrophy may be sought in a direct effect of the thyroid hormones produced in increased amounts [25]. As a result of the enhanced mineral absorption, the serum Ca level increases [21], and this results in depression of parathyroid hormone (PTH) secretion [7, 22], while leaving the sensitivity of the tissues to PTH unaffected [12]. These changes go hand in hand with a fall in the serum-1,25-dihydroxy-vitamin D_3 concentration, which accounts for the impairment of Ca absorption from the intestine in thyrotoxicosis [18]. All this contributes to the reduction of the cancellous and cortical bone mass [23].

The question how calcitonin (Ct) behaves in thyrotoxicosis has yet to be studied. While according to earlier data the basal level is within the normal

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range [1, 14], our studies are suggestive of a Ct hypersensitivity of the tissues in this disease [13] which may result from an increased rate of bone metabolism [3].

The present study has been concerned with the following questions: [1] Is there any relationship between the fall in bone mineral content and the serum thyroid hormone levels in thyrotoxicosis? [2] To what degree is the serum Ct level affected in the various types of thyrotoxicosis (Graves' disease, destructive hyperthyroidism). [3] In acute thyroid damage consecutive to radioiodine therapy is Ct released from the thyroid in sufficient amounts to modify the serum Ct and Ca levels? [4] Are the Ca and Ct responses to Ca loading different in euthyroid and hyperthyroid subjects?

Materials and methods

Euthyroid and hyperthyroid females aged between 20 and 50 years were studied. The clinical diagnosis was confirmed on the basis of the serum thyroxine (T_4) and triiodothyronine (T_3)level. For T_4 measurement the RK 12 kit, for that of T_3 the RK 11 kit (Isotope Institute, Hungarian Academy of Sciences) was used. Bone mineral content was measured with a Norland-Cameron BMA 178 type instrument, operating on the Cameron-Sorensen principle. The measurements were done on the non-dominant side of the radius, between the middle and distal third. Four parallel measurements were done on each occasion and the values were averaged. The instrument gives the mineral content in g/cm and the width of the bone in cm [16, 27].

Se total Ca was estimated with the Unicam SP 190 atomic absorption spectrophotometer. Since hyperthyroid subjects are liable to hypoalbuminaemia [8], the basal se Ca values (mmol/l) were corrected in each case for se albumin (mmol/l) individually, according to Mosekilde et al. [24]. (Corrected se Ca = $1.2 \times$(0.700-se albumin) + total se Ca.) Though the basal se Ca levels in the thyrotoxic cases were found between normal limits (2.25–2.65 mmol/l), the corrected se Ca value was higher in the hyperthyroid than in the euthyroid group.

For the measurement of serum Ct, the Immune Nuclear Corporation's calcitonin II human radioimmunoassay kit was used (ref. standard: W.H.O. 70/234). The sensitivity of the procedure was 15 pg/ml, intraassay variation coefficient was 5%.

Eight euthyroid and seven thyrotoxic female patients kept on a low Ca diet, were subjected to Ca loading in the morning after an overnight fast; 3.64 mg/kg (0.2 ml/kg) of a 10% solution of $CaCl_2$ was administered i.v. in 3 min. Blood was withdrawn for Ca and Ct studies prior to and at 5, 10 and 60 min after the injection. From six patients with Graves' disease blood was withdrawn also for Ca and Ct studies prior to and at 24 and 120 h after radioiodine therapy (190–300 MBq ^{131}I). The sera were stored at —20 °C until study.

For statistical analysis Student's two sample *t* test was used. In the Tables the mean values ±S.E.M. are represented.

Results

Table I shows the mean mineral content of the radius in 24 euthyroid cases and in 28 patients with Graves' disease. The values were significantly lower in the patients with Graves' disease than in those with a normal thyroid ($P < 0.05$). On examining the patients with thyroid disease individually, 12 of 28 women with Graves' disease (43%) were found to have reduced osteodensitometric values. Grouped on the basis of the T_3 level, in the group marked by a significant T_3 elevation (4.6–7.0 nmol/l) the mineral content of radius was significantly lower than in the euthyroid cases ($P < 0.01$). On the other

hand, no difference in mineral content of the radius was found between the patients with minor hyperthyroidism and the euthyroid group (Table II). No typical relationship was, however, found between the mineral content of the radius and the serum T_4 level in Graves' disease.

The basal Ca value corrected for albumin, and the Ct value in the euthyroid subjects and the patients with Graves' disease and subacute thy-

Table I

Mineral content of radius (mean ± SEM) in females aged 20–50 years with normal thyroid function and with Graves' disease

	Mineral content of radius
Euthyroidism (n = 24)	0.859 ± 0.014
Graves' disease (n = 28)	0.797 ± 0.020
P	< 0.05

Table II

Mean bone mineral content and serum triiodothyronine level in females with euthyroidism and with Graves' disease (mean ± SEM)

	Hyperthyroidism T_3 : 3.1—4.5 nmol/l n = 15	P	Euthyroidism T_3 : 1.2—3.0 nmol/l n = 24	P	Hyperthyroidism T_3 : 4.6—7.0 nmol/l n = 13
Mineral content of radius g/cm	0.820 ± 0.024	N.S.	0.859 ± 0.014	< 0.01	0.772 ± 0.032

N.S. = non-significant

Table III

Basal serum Ca (corrected, see text) and calcitonin concentration in females with euthyroidism, Graves' disease and subacute thyroiditis (mean ± SEM)

	Serum Ca mmol/l (corrected)		Basal serum calcitonin pg/ml
Euthyroidism (n = 17)	2.58 ±0.03	P < 0.01	95.71 ±8.56
Graves' disease (n = 15)	2.73 ±0.03		97.73 ±8.91
Subacute thyroiditis (n = 6)	2.60 ±0.02		99.81 ±9.98

Table IV

Serum calcium and calcitonin level in response to i.v. $CaCl_2$ loading (3.64 mg/kg/3 min) in females with euthyroidism and Graves' disease (mean ± SEM)

	Serum calcitonin, pg/ml				
	0 min	5 min	10 min	30 min	60 min
Euthyroidism (n = 8)	86.6 ±7.75	129.1 ±11.29	118.6 ±10.10	105.2 ±6.10	96.0 ±6.57
Graves' disease (n = 7)	87.1 ±7.22	125.7 ±11.46	119.1 ±8.91	101.1 ±8.92	91.4 ±8.37

	Serum calcium, mmol/l				
	0 min	5 min	10 min	30 min	60 min
Euthyroidism (n = 8)	2.35 ±0.05	2.88 ±0.08	2.80 ±0.07	2.74 ±0.03	2.61 ±0.05
Graves' disease (n = 7)	2.44 ±0.01	2.89 ±0.07	2.80 ±0.07	2.73 ±0.06	2.68 ±0.03

Table V

Serum calcitonin and Ca concentration after radioiodine therapy (n = 6) (mean ± SEM)

Time h	Serum calcitonin pg/ml		Serum Ca mmol/l
0	97.33 ± 6.30		2.47 ± 0.059
24	94.20 ± 6.99	$P < 0.01$	2.48 ± 0.061
120	70.30 ± 5.58		2.50 ± 0.094

roiditis are shown in Table III. The se Ca level corrected for the albumin content was significantly higher in Graves' disease than in the case of normal thyroid function ($P < 0.01$). The basal serum Ct concentration was identical in all the three groups.

Table IV shows the results of calcium loading. The response of the se Ca and Ct concentration was identical in the euthyroid and the thyrotoxic groups.

Table V presents the se Ca and Ct values prior to and at 24 and 120 h after radioiodine therapy in the thyrotoxic patients. At 120 h after ^{131}I therapy the se Ct concentration was significantly lower than the initial value ($P < 0.01$) while the se Ca level was unchanged.

Discussion

The observations relating to the frequency and degree of bone mineral loss in hyperthyroidism are inconsistent. While Frazer et al. [15] and Ikkos et al. [19] found a loss mainly in women of advanced age other authors reported on mineral loss in a higher proportion of patients [5, 18], regardless of age and sex. Photon absorption over the radius indicates not only the mineral mass of the forearm bones, but also of the entire skeletal system [11]. Our results agree well with those of Linde and Friis [20] according to which the mean mineral content of the radius is lower in hyperthyroid than in euthyroid subjects. Bone abnormalities of this type were found in 42.8% of hyperthyroid females between 20 and 50 years of age, particularly if the se T_3 level was in excess of 4.6 nmol/l. This again is consistent with the observation that the disturbed mineral metabolism in hyperthyroidism correlates well with the severity of the disease, reflected in the serum T_3 level [21].

PTH secretion is known to be depressed in thyrotoxicosis [7, 22]. The other hormone involved in bone metabolism, calcitonin, has been studied less extensively although its physiological role in humans is still controversial [3], though it has been ascertained that in pharmacological doses it inhibits osteoclast active bone absorption, that it interferes with the reabsorption of Ca and P in the kidney and that it acts on the intestines, liver and pancreas.

The parafollicular cells of the thyroid form the main site of Ct production in man. Thyroid agenesis, congenital nongoitrous cretinism [10] and post-thyroidectomy state [26] are associated with a reduction of the se Ct level. Yet hypocalcaemia remains absent and the organism readily adapts itself to the Ct deficiency. In Graves' disease the basal se Ct level was found to be normal [14] in contrast to destructive hyperthyroidism (Hashimoto's thyroiditis, subacute thyroiditis), in which an elevation of the se Ct level due to excessive release was observed [1]. Our studies have failed to demonstrate any significant difference in the se Ct level between thyrotoxic patients with Graves' disease, those with subacute thyroiditis and the normal controls.

In hyperthyroidism even a minor hypercalcaemia is sufficient for the depression of PTH secretion [8]. On the other hand, a minor increase in the Ca level does not seem to be sufficient to increase Ct secretion. It is well within the possibilities that in this disease the parafollicular cells fail to respond adequately to minor variations of the serum Ca level. Finally, in hyperthyroidism an accelerated breakdown may perhaps account for the normal basal Ct level. It seemed, therefore justified to examine these possibilities by studying the Ct and Ca response to Ca loading, the more so as no such studies have been performed in Graves' disease, while in nongoitrous cretinism and in thyroidectomized subjects Ca loading was followed by a delayed clearance of Ca from the blood [10, 17]. On the evidence of the present study, Ca loading

was followed by an equal increase and subsequent decline of the serum Ca and Ct in hyperthyroid and euthyroid cases alike. The Ct reserves thus proved to be identical in both groups. The parafollicular cells of thyrotoxic patients thus respond to variations in the se Ca level in the same manner as do those of normal subjects.

Our results in subacute thyroiditis are at variance with the recent observations reported by Amino et al. [1]. In order to find out whether or not an acute damage to the thyroid is associated with an increased liberation of Ct sufficient to cause transitory changes in the serum Ca and Ct levels, we have studied them prior to and at 24 and 120 h after radioiodine therapy. The study was prompted by the following considerations. The transitory hypocalcaemia consequent upon subtotal thyroidectomy has been connected with manipulations on the gland resulting in an enhanced release of Ct [30]; and there is experimental evidence to suggest that the parafollicular cells are more sensitive to radiation than are the epithelial cells [28]. According to our studies, after irradiation of the thyroid with approx. 7000 rad (70 Gy), the gland not only fails to release an increased amount of Ct but on the 5th day after ^{131}I treatment the Ct level was below the initial value. Though subacute thyroiditis and radioiodine therapy do not produce the same type of thyroid injury, our results make it none the less unlikely that in subacute thyroiditis the destructive lesion should produce a Ct release sufficient to increase the serum Ct level. We have no explanation for this fall in serum Ct but it might be attributed to the high radiosensitivity of the parafollicular cells [28], as a result of which the production of Ct might be affected by radiation. It also emerged from our results that despite the acute fall in the serum Ct level, the serum Ca concentration was unaffected. This leaves us with the questions whether the depression of Ct secretion after radioiodine therapy is persistent, whether the responsiveness of the parafollicular cells is affected and, if so, whether the changes involve late consequences to the skeletal system. Further studies will have to throw light on these questions.

Acknowledgement

We are indebted to the laboratory assistants, Mrs. J. Juhász and Miss I. Jókai for skilful help.

REFERENCES

1. Amino, N., Yabu, Y., Miki, T., Morimoto, S., Kumahara, Y., Mori, H., Iwatani, Y., Nishi, K., Nakatani, K.: Serum ratio of T_3 to T_4 and TBG and calcitonin concentrations in Graves' disease and destruction induced thyrotoxicosis. J. Clin. Endocr. **53**, 113–116 (1981).
2. Anast, C. S., Guthrie, R. A.: Decreased Ca tolerance in nongoitrous cretins. Pediat. Res. **5**, 668–672 (1971).
3. Austin, L. A., Heath, H.: Calcitonin. New Engl. J. Med. **304**, 269–278 (1981).

4. Baylin, S. B., Bailey, A. L., Hsu, T. H., Foster, G. V.: Degradation of human calcitonin in human plasma. Metabolism **26,** 1345–1348 (1977).
5. Bailey, T. A., Harrison, J. E., McNeill, K. G., Mernagh, J. R.: Effect of thyrotoxicosis and its treatment on bone mineral and muscle mass. J. Clin. Endocr. **50,** 916–922 (1980).
6. Bouillon, R., Muls, E., De Moor, P.: Influence of thyroid function on the serum concentration of 1, 25-dehydroxy-vitamin D_3. J. Clin. Endocr. **51,** 793–797 (1980).
7. Bouillon, R., De Moor, P.: Parathyroid function in patients with hyper or hypothyroidism. J. Clin. Endocr. **38,** 999–1007 (1974).
8. Burman, K. D., Monchik, J. M., Earll, J. M., Vartofsky, L.: Ionised and total serum Ca and parathyroid hormone in hyperthyroidism. Ann. Intern. Med. **86,** 668–671 (1976).
9. Cameron, J. R., Sorensen, J.: Measurement of bone mineral in vivo: An improved method. Science **142,** 230–232 (1963).
10. Carey, D. E., Jones, K. L., Parthemore, J. G.: Calcitonin secretion in congenital nongoitrous cretinism. J. Clin. Invest. **65,** 892–895 (1980).
11. Cohn, S. H., Ellis, K. J., Goldsmith, N. F.: Validity of the absorbtiometric measurement of bone mineral content of the radius. Amer. J. Roentg. **126,** 1286–1292 (1976).
12. Földes, J., Gyertyánffy, G., Juhász, J.: PTH terhelés hatása a vizelet cAMP ürítésre euthyreosisban és hyperthyreosisban (Effect of PTH loading on urinary cAMP excretion in euthyroidism and hyperthyroidism). Magy. Belorv. Arch. **31,** 252–255 (1978).
13. Földes, J., Bános, Cs.: Altered calcitonin sensitivity in thyroid disorders. Horm. Metab. Res. **12,** 283–284 (1980).
14. Frazer, S. A., Wilson, G. M.: Plasma calcitonin in disorders of thyroid function. Lancet **1,** 725—726 (1971).
15. Frazer, S. A., Anderson, J. D., Smith, D. A., Wilson, G. M.: Osteoporosis and fractures following thyrotoxicosis. Lancet **1,** 981–984 (1971).
16. Gergely, I., Krasznai, I., Horváth, T., Szücs, J., Holló, I.: Egészséges felnőttek radiusának ásványianyag-tartalma (Mineral content of the radius of healthy adults). Orv. Hetil. **119,** 699–703 (1978).
17. Grubb, S. A., Decker, S. A., Taft, T. N., Talmage, R. N.: Effect of long-term thyroidectomy on post-prandial plasma Ca changes in man. J. Endocr. Invest. **2,** 75–78 (1979).
18. Haldimann, D., Kaptein, E. M., Singer, F. R., Nicoloff, J. T., Massry, S. G.: Intestinal Ca absorption in patients with hyperthyroidism. J. Clin. Endocr. **51,** 995–997 (1980).
19. Ikkos, D. G., Katsichtis, P., Natalles, K., Valentzas, G.: Osteoporosis in thyrotoxicosis. Lancet **2,** 1159–1161 (1971).
20. Linde, J., Friis, Th.: Osteoporosis in hyperthyroidism estimated by photon absorptiometry. Acta Endocr. (Kbh.) **91,** 437–448 (1979).
21. Manicourt, D., Demeester-Mirkine, N., Brauman, H., Corvilain, J.: Disturbed mineral metabolism in hyperthyroidism: Good correlation with T_3. Clin. Endocr. **10,** 407–412 (1979).
22. Mosekilde, L., Christensen, M. S.: Decreased parathyroid function in hyperthyroidism. Acta Endocr. (Kbh.) **84,** 566–573 (1977).
23. Mosekilde, L., Melson, F., Bagger, J. P., Myre-Jensen, O., Sorensen, N. S.: Bone changes in hyperthyroidism. Acta Endocr. (Kbh.) **85,** 515–525 (1977).
24. Mosekilde, L., Christensen, M. S., Melsen, F., Sorensen, N.: Effect of antithyroid treatment on Ca-P metabolism in hyperthyroidism. Acta Endocr. (Kbh.) **87,** 743–750 (1978).
25. Mundy, G. R., Shapiro, J. L., Bandelin, J. G., Canalis, E. M., Rais, L. G.: Direct stimulation of bone resorption by thyroid hormones. J. Clin. Invest. **58,** 529–534 (1976).
26. Silva, O. L., Wisneski, L. A., Cyrus, J., Snider, R. H., Moore, C. F., Becker, K. L.: Calcitonin in thyreoidectomised patients. Amer. Med. Sci. **275,** 159–164 (1978).
27. Szücs, J.: Calcitonin és az uraemiás csontdystrophia (Calcitonin and uraemic osteodystrophy). C. Sc. thesis, Budapest 1977. p. 46.
28. Thurson, V., Williams, E. D.: The effect of radiation on thyroid C cells. Acta Endocr. (Kbh.) **99,** 72–78 (1982).
29. Valentzas, C., Oreopulos, D. G.: Thyrotoxic osteodystrophy. Clin. Invest. Med. **1,** 53–58 (1978).
30. Watson, Ch. G., Steed, D. L., Robinson, A. G., Deftos, L. J.: The role of calcitonin and PTH in the pathogenesis of post-thyroidectomy hypocalcaemia. Metabolism **30,** 588–589 (1981).

Acta Medica Hungarica 40 (2—3), pp. 107—124 (1983)

MANAGEMENT OF HORMONE-SECRETING PITUITARY MICROADENOMAS

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(Received October 5, 1982)

Experience with 22 patients with pituitary microadenoma is discussed. The diagnosis was based on examination of the contour of the normally sized sella turcica by multidirectional thin-layer tomography, and measurement of the pituitary hormone reserve capacity. Of the patients 13 had a microprolactinoma and 9 a microsomatotropinoma; 13 were subjected to trans-sphenoidal microadenomectomy and 14 received drug treatment. The problems of diagnostics and the choice of therapy are discussed.

Keywords: pituitary microadenomus, bromocriptine therapy

Abbreviations

ACTH = adrenocorticotrophin, FSH = follicle-stimulating hormone, hGH = = human growth hormone, LH = luteinizing hormone, LH–RH = luteinizing hormone-releasing hormone, PRL = prolactin, T_3 = triiodothyronine, T_4 = thyroxine, TSH = thyrotropin, TRH = thyrotropin-releasing hormone

Introduction

Hormone-secreting pituitary adenomas are discrete nodules of the adenohypophysis; they are called microadenoma when they are less than 10 mm in diameter [24]. Post mortem examinations have shown that the prevalence of microadenomas lies in the range of 2.7–22.5% [12, 24]. Active microadenomas produce increased amounts of one or several pituitary hormones, which cause clinical symptoms of acromegaly, amenorrhoea-galactorrhoea syndrome and Cushing's or Nelson's syndrome [13, 38, 42]. The tumours are named according to the hormone produced. Radioimmunoassay for human growth hormone (hGH), prolactin (PRL) and adrenocorticotrophin (ACTH) can be used to evaluate pituitary secretory function in patients with these clinical symptoms; adenomas that secrete thyrotropin (TSH) or gonadotropin are extremely rare. Endocrine assessment requires exact documentation of hormonal hypersecretion and determination of the pituitary reserve capacity of the other hormones. Endocrine-inactive microadenomas do not show symptoms of hormone secretion on either clinical examination or endocrine testing.

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Of the non-invasive radiological investigations available, a plain lateral skull X ray cannot be considered conclusive by itself. While the finding of an abnormal sella does not exclude other conditions, its most likely cause is an expanding pituitary tumour. Similarly, a normal fossa in a patient with clinical and hormonal evidence of a pituitary adenoma may well harbour a microadenoma. In the latter case, multiple thin-layer multidirectional tomography of the sella has been advocated in an attempt to identify such a lesion. The asymmetrical bulging of the sella floor in these patients, is revealed only by this method [52].

For the treatment of pituitary microadenomas, three kinds of therapy are available, viz. surgery, irradiation and drugs.

Trans-sphenoidal approach to the sella turcica has supplanted intracranial exploration for pituitary adenomas [21] except when the tumour had invaded the brain substance [22, 43]. With the use of the operating microscope and image amplification, this approach permits easy access to the sphenoid sinus and the floor of the sella [25, 53]. Recent reports show that removal of microadenomas by a neurosurgeon experienced in microsurgery may produce improvement of the clinical and hormonal abnormalities in most patients [45].

Conventional external irradiation of the pituitary has frequently received criticism for its delayed effect in controlling the growth of pituitary adenoma; in addition, it has been associated with unacceptable and unpredictable side-effects [33]. Proton or other heavy-particle irradiation is advocated as initial therapy by some authors because it allows to deliver high doses to confined intrasellar tumours, with reduction of hypersecretion, preservation of other pituitary functions, and minimal risk of hypothalamic damage [32]. Radiation treatment continues to be important when surgical removal is incomplete or the tumour recurs.

Of major importance in recent years has been the development of effective drugs that suppress hormone secretion [5, 6, 7, 9, 10, 14, 28, 30, 36, 37, 50]. A reasonably well-defined field of indication of the medical treatment of pituitary microadenomas has lately emerged: such treatment is necessary if [1] a selective pituitary effect is required (e.g. for the maintenance of follicle stimulating hormone (FSH) and luteinizing hormone (LH) production in patients of fertile age); [2] there is a need for postoperative or postirradiation decrease of hormone production; [3] it is desired to attain a preoperative antiproliferative effect; and [4] in the case of pituitary diseases of assumed hypothalamic origin [19].

Endocrine-active microadenomas may grow and become endocrine macroadenomas. Because of this possibility the early diagnosis of pituitary tumours is important. In the following, a review will be given of the endocrine, radiological and therapeutic advances in the management of pituitary microadenomas on the basis of our clinical and laboratory experience.

Patients and methods

In the period 1978–82, 22 patients were observed, 18 patients were female, and 4 male; their age ranged from 22 to 60 years. The duration of the disease was from 2 to 24 years. Of these patients, 13 were subjected to trans-sphenoidal adenomectomy.

Of the patients, 13 had hypogonadism (raromenorrhoea, amenorrhoea, sterility) and galactorrhoea. One male patient (patient 1) had gynaecomastia, and a female patient (patient 2) complained of dry skin and bradycardia. Nine patients showed acromegaly with excessive sweating and headaches (Table I).

Table I

Clinical data of patients with microadenoma

No.	Age (years)	Sex	Clinical symptoms	Duration of disease (year)	Sella		Visual field
					mm²	shape	
1	28	M	hypogonadism, gynaecomastia, obesity	8	90	bulging	enlarged blind spot
2	35	F	amenorrhoea, dry skin, bradycardia	8	110	sloping	enlarged blind spot
3	28	F	amenorrhoea, dwarfism	6	110	sloping	normal
4	31	F	amenorrhoea, galactorrhoea	4	90	bulging	normal
5	30	F	galactorrhoea	3	70	bulging	normal
6	22	F	galactorrhoea	3	90	erosion	normal
7	34	F	amenorrhoea	4	100	sloping	normal
8	31	F	infertility	3	80	bulging	normal
9	30	F	raromenorrhoea, galactorrhoea, infertility	5	70	sloping	normal
10	25	F	amenorrhoea, galactorrhoea	4	80	bulging	normal
11	31	F	amenorrhoea, galactorrhoea	7	90	bulging	normal
12	31	F	raromenorrhoea	3	120	erosion	normal
13	29	F	infertility	3	80	bulging	normal
14	47	M	acromegaly, hyperhydrosis, headache	24	90	bulging	normal
15	35	F	acromegaly, headache	7	110	sloping	normal
16	47	M	acromegaly,	14	110	bulging	normal
17	53	M	acromegaly, hyperhydrosis, headache	20	110	erosion	normal
18	43	F	acromegaly	2	70	bulging	normal
19	46	F	acromegaly, hyperhydrosis, headache	6	80	erosion	normal
20	60	F	acromegaly, headache	20	80	sloping	normal
21	56	F	acromegaly, headache hyperhydrosis	3	80	sloping	normal
22	54	F	acromegaly, headache, hyperhydrosis	3	110	erosion	normal

Diagnostic procedures

Radiological investigation. A plain skull radiograph was made in every case. After evaluation of the size and contours of the sella turcica, double-directional and multi-level thin-layer tomography (2 mm) was performed. In acromegalic patients, X-rays of the hands and legs were made.

Endocrinologic investigation. Basal hormone measurements alone provide insufficient evidence to diagnose anterior pituitary disease (hyper- and hyposecretion). A differential diagnosis may be possible between primary and secondary (hypothalamic) pituitary failure on a theoretical basis. TSH and PRL were measured 0, 30, 60, 90 and 120 min after intravenous administration of 200 μg synthetic TSH-releasing hormone (TRH, Relefact, Hoechst). LH and FSH were measured 0, 30, 60, 90 and 120 min after intravenous administration of 25 μg synthetic LH-releasing hormone (LH-RH, Relisorm, Serono). The serum hGH was measured in acromegalic patients during an oral glucose tolerance test. hGH, PRL, FSH and LH were determined by radioimmunoassay using Serono-Biodata Kits.

Blood glucose was determined with the *o*-toluidine technique, serum cortisol by a fluorometric method. Hormone measurements were performed before and 1–2 weeks after surgery and during medical treatment.

Treatment

Surgery. Of the trans-sphenoidal approaches to the sella we used a variant of the lower nasal form [44], the transnasal-trans-septal-trans-sphenoidal approach. Operations were performed using microsurgical instruments and microscope.

Irradiation. Patients received fractional telecobalt irradiation to the hypophyseal area in a dose of 40 Gy.

Medical treatment. Hyperprolactinaemic patients received bromocriptine (Parlodel, Sandoz) in a dose of 7.5 mg daily for 4–6 months. Acromegalic patients received the same drug in a dose of 10 mg daily for 4–12 months.

Histological examination. Electron microscopic examination was performed after adenomectomy in all of the operated patients. In three patients an immunocytochemical light micrograph was made.

Results

All 22 patients with pituitary adenoma had a sella of normal area (130 mm^2). Thin-layer tomograms of the sella demonstrated abnormal configurations. These changes were in 16 cases noted in lateral tomograms: bulging or localized erosion of the sella was usually present along the anterior or anteroinferior margin. On frontal tomograms the floor of the sella was straight or slightly convex inferiorly. Microadenomas depress the floor on one side, which is called the "sloping" sign; this could be observed in seven patients (Figs 1, 2).

X-rays of the hands showed marked thickening of soft tissues, widened bones, periosteal reaction, small osteophytes, tufting and mushrooming of the terminal phalanges.

The visual field was normal in all but two patients. In these latter cases an enlarged blind spot could be observed (Table I). Tables II and III show the laboratory data; the values *a* are basal values, while the *b* values were measured after various stimulations.

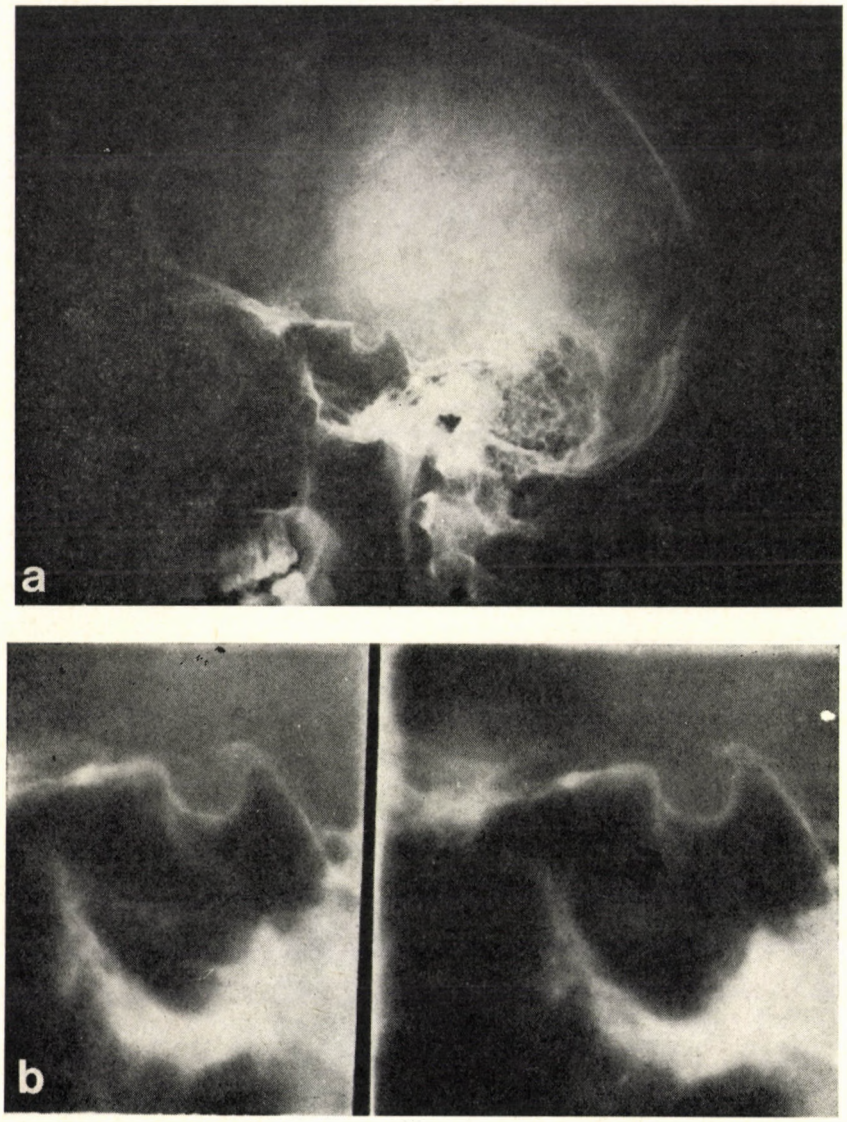
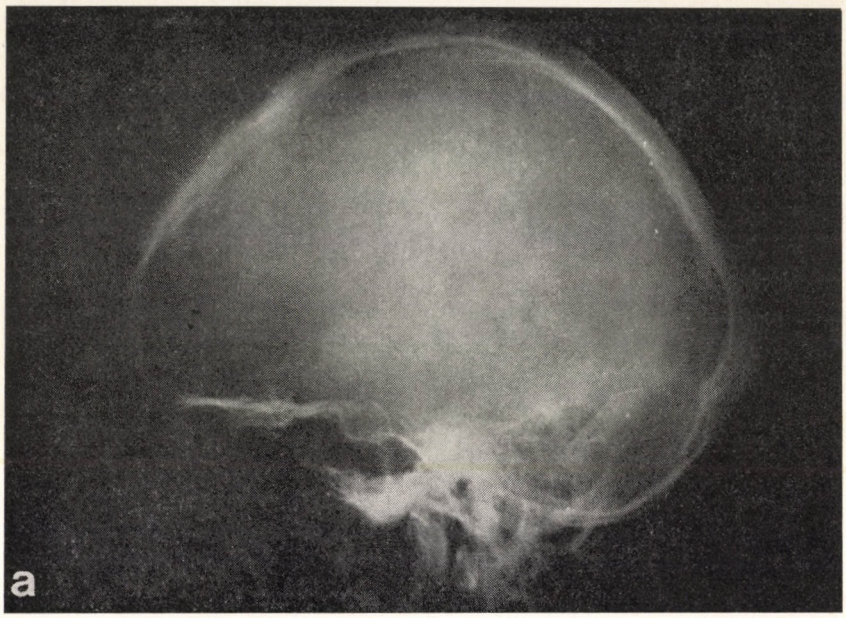


Fig. 1. Lateral skull X-rays and sagittal tomogram of the sella of patient 3. *a* The lateral film of the sella shows no abnormality. *b* Localized bulging (arrow) of the sella floor is visible

An elevated serum PRL level could be detected in 13 patients, one of whom (patient 17) had acromegaly. A female patient with galactorrhoea (patient 5) had a normal basal serum PRL level, but a pathological reserve capacity was shown during the TRH test. The serum basal FSH and LH were highly elevated in a male patient with gynaecomastia (patient 1); chromosome analysis showed a karyogram of the mosaic Klinefelter's syndrome: 46 XY/47



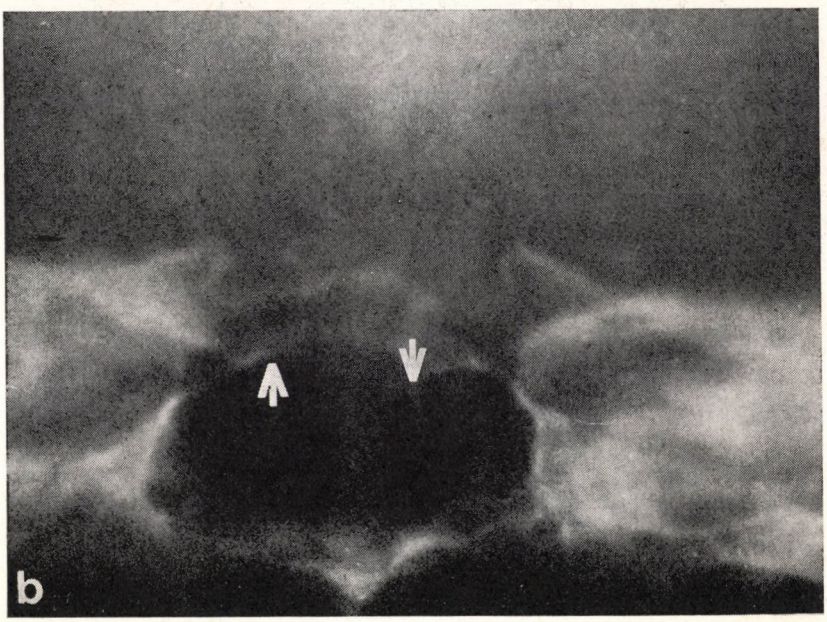


Fig. 2. Lateral skull X-ray and frontal tomogram of the sella of patient 6. *a* The lateral film shows a sella of normal size. *b* Sloping sign on the frontal tomogram

Table II

Laboratory data of patients with microadenoma, I

No.	Before treatment					Treatment	After treatment				
	PRL mU/l	FSH U/l	LH U/l	TSH mU/l	Cortisol nmol/l		PRL mU/l	FSH U/l	LH U/l	TSH mU/l	
1a	2496	45	118	4.4	474	—	—	—	—	—	karyogram
b		86	460	14.5							46XY/47XXY
2a	2628	5.2	8.3	420	385	adenomectomy	1173	1.7	6.5	4.5	T_3 0.2 nmol/l, T_4 36 nmol/l
b		7.2	11.6	630		triiodothyronine		5.8	13.0	14.0	
3a	36,980	9.2	9.4	2.5	180	Parlodel	2430				
b		15.0	25.0	19.8		adenomectomy a	14,180	2.3	4.5	5.3	
						b		3.0	14.0	21.0	
4a	4492	5.7	21.0	7.0	238	adenomectomy a	786	3.8	7.6	4.1	
b		37.0	171	22.0		b		4.9	9.7	8.5	
5a	320	—	—	—	324	adenomectomy a	230	—	—	—	
b	14,520					b	2680				
6	11,636	—	—	—	286	adenomectomy	1125	—	—	—	
7	1376	—	—	—	328	adenomectomy	676	—	—	—	
8	5728	—	—	—	234	Parlodel	604	—	—	—	
9a	7980	7.6	5.3	3.3	464	Parlodel	654	—	—	—	
b		18.6	66								
10	18,240	3.3	8.2	3.2	435	Parlodel	3244	—	—	—	
11a	4,865	2.0	40.8	1.2	342	adenomectomy	1380	3.5	1.6	3.2	
b		6.0	66.0	6.5		Parlodel	205	13.0	50.0	7.1	
12a	2,877	5.1	9.1	2.3	345	adenomectomy a	831	5.5	5.3	5.6	
b		10.0	30.0	4.5		b		8.0	19.0		
13a	3,896	1.7	11.5	2.2	453	Parlodel	642	—	—	—	
b		4.0	107.3	18.3							

Table III

Laboratory data of patients with microadenoma, II

No.	Before treatment						Treatment	After treatment				
	hGH pmol/l	FSH U/l	LH U/l	TSH mU/l	PRL mU/l	Cortisol nmol/l		hGH pmol/l	FSH U/l	LH U/l	TSH mU/l	PRL mU/l
14a	674	10	12	6.5	292	246	Parlodel	395	—	—	—	—
b		—	—	20.5	1462		adenomectomy	204	3.9	3.2	4.0	211
15	1646	—	—	—	—	326	Parlodel	576	—	—	—	—
16a	2790	19	3	6.5	—	345	Parlodel	860	16.0	0.6	—	—
b		22	14	22			adenomectomy	223	20.0	10	—	—
17a	2325	2.4	7.8	3.3	1137	446	irradiation	1841	4.3	6.5	1.5	1486
b		8.5	20	8.5	1625		Parlodel	1138	6.1	19.2	2.4	680
18a	804	—	—	2.4	585	343	Parlodel	437	—	—	1.6	37
b				12.5	2115		adenomectomy	97			14.3	356
19a	4324	90	110	2.4	136	346	irradiation	6324	86	101	—	517
b		—	—	2.7	533		Parlodel	1367	—	—	—	982
20a	1906	15	19	2.2	—	424	Parlodel	1534	—	—	1.6	—
b		30	52	8.6	—		adenomectomy a	394	—	—	7.2	73
							b					344
21a	376	44	65	2.5	158	234	Parlodel a	120	—	—	3.5	324
b		94	278	12.9	849		b		—	—	13.6	771
22a	1748	88	139	9.8	503	423	Parlodel	2148	48	93	—	—
b		134	530	13	640		adenomectomy a	423	29	28	2.1	169
							b		34	61	1.7	344

XXY. Further 2 patients (patients 21, 22) had slightly elevated FSH and LH levels due to menopause.

The serum TSH level was within the normal range in 16 patients. Patient 2 had a highly elevated TSH level due to primary hypothyroidism; this was proved also by measurement of the T_3 and T_4 levels.

The serum hGH level could not be suppressed below 232 pmol/l: during glucose loading the acromegalic patients were all in the active stage of the disease. The serum cortisol level proved to be normal in all patients. X rays and hormone determinations led to a diagnosis of microprolactinoma in Cases 1–13 and hGH-producing microadenoma in Cases 14–22.

Five patients with prolactinoma received preoperative bromocriptine treatment. In three of these cases (patients 3, 9, 10) regular menstrual bleeding occurred and the galactorrhoea diminished. The serum PRL level was lowered in two (patients 3, 10) and normalized in three cases (patients 8, 9, 13). The serum PRL decrease appeared within one week after the beginning of bromocriptine therapy. Eight patients with prolactinoma underwent transsphenoidal microsurgery. In two cases (patients 4, 5) the galactorrhoea ceased, while in further two cases (patients 6, 11) it diminished markedly. In four operated cases regular menstrual bleedings were elicited by bromocriptine treatment (patients 2, 7, 11 and 12). The serum PRL level normalized in four (patients 4, 5, 7, 12) and greatly diminished in the remaining four cases (patients 2, 3, 6, 11). In patient 11, postoperative bromocriptine treatment normalized the serum PRL level; the remaining three patients with moderately elevated PRL levels are candidates for postoperative drug treatment (Fig. 3).

The serum TSH level of patient 2 could be lowered by triiodothyronine substitution after the operation. The TSH reserve capacity and serum cortisol were normal in all of the acromegalic patients before treatment.

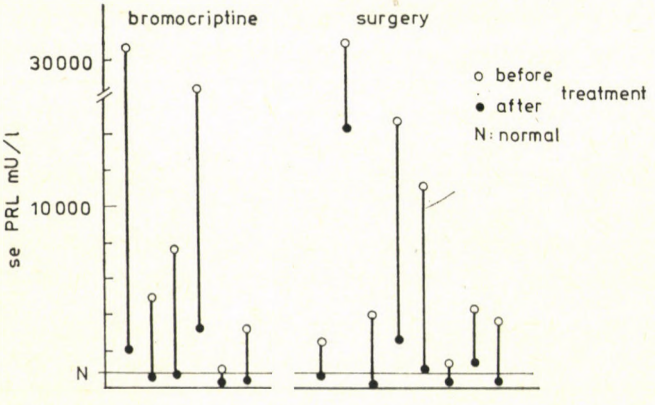


Fig. 3. Effect of bromocriptine and surgical treatment on the PRL level of patients with microprolactinoma

Seven acromegalic patients received bromocriptine treatment for 4–12 months. Under its effect, five patients reported on improvement of their general condition, in four patients the headaches had diminished and in four cases the digital circumference decreased while 6 patients complained of constipation as a side-effect and in one case was aggravated the anaemia. The serum hGH level was lowered in six patients and had normalized in a

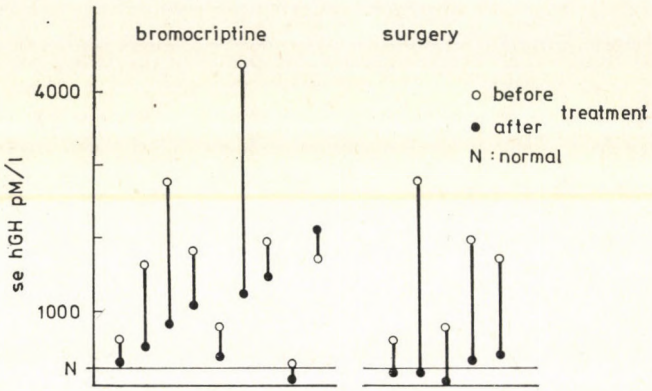


Fig. 4. Effects of bromocriptine and surgical treatment on the hGH level of acromegalic patients

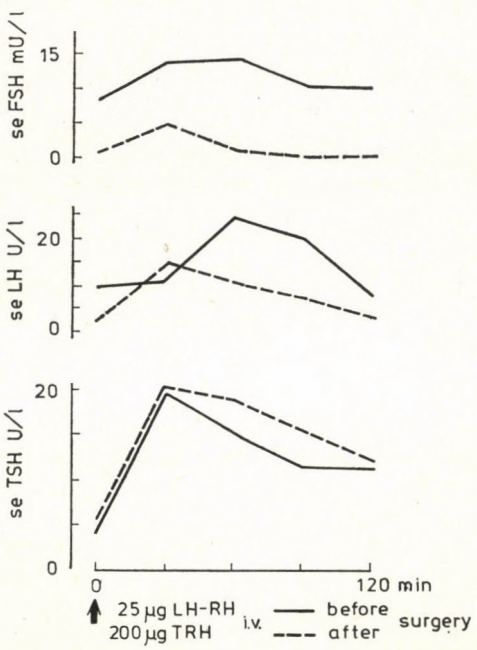


Fig. 5. Anterior pituitary hormone reserve capacity of patient 3 before and after surgical treatment

single case. The decrease appeared 2–3 weeks after the beginning of treatment while in one patient (patient 22) a moderate hGH elevation was seen during bromocriptine therapy. Two patients who had refused surgical treatment received telecobalt irradiation of the pituitary. The elevated serum hGH persisted 2–3 months after the irradiation and bromocriptine treatment was required. Five acromegalics were subjected to trans-sphenoidal adenomectomy, which was followed by clinical improvement. In three of them (patients 14, 16, 18) a normal hGH level could be measured after removal of the microadenoma; the remaining two patients showed a marked decrease of the hGH level (Fig. 4). Drug treatment, surgical intervention or irradiation could improve the gonadotropin and TSH reserves in only two of the patients. The TSH reserve capacity even deteriorated in patient 17 after telecobalt irradiation, and the same could be observed after adenomectomy in patient 22 (Fig. 5).

Postoperative complications. There was no postoperative complication in this series of patients.

Histological findings

Electron microscopic examination was performed on all removed adenomas. The findings supported the clinical diagnosis in all cases: eight of the adenomas proved to be PRL, four of them GH cell and one of them a mixed GH–PRL cell pituitary adenoma. In three of the operated cases light microscopic immunohistochemical examination was also performed (Figs 6, 7, 8).

Discussion

Pituitary adenoma is a benign neoplasm with autonomous growth or autonomous function, or both. The autonomy need not be absolute; a certain degree of hypothalamic influence may persist for a time, but the dependence is lost gradually during the evolution of the proliferative process [31]. Microadenomas are able to produce pituitary hormones in excess which may cause clinical symptoms and may be detected in the blood. In 13 of our cases the microprolactinoma led to galactorrhoea-amenorrhoea and hypogonadism, and in nine patients the microsomatotropinoma caused acromegaly. By the definition of Hardy, microadenomas measure 10 mm or less in diameter and are usually located within the sella [24].

The normal pituitary may undergo a number of structural changes. The aetiology of these processes is mostly unknown, but recent observations have shown that hormonal changes e.g. pregnancy, oestrogen treatment or hypothyroidism, by a direct or indirect (hypothalamic) action may elicit a diffuse hyperplasia of a particular cell type in the anterior pituitary. The endocrine-active microadenoma may be caused by peripheral hormone dis-

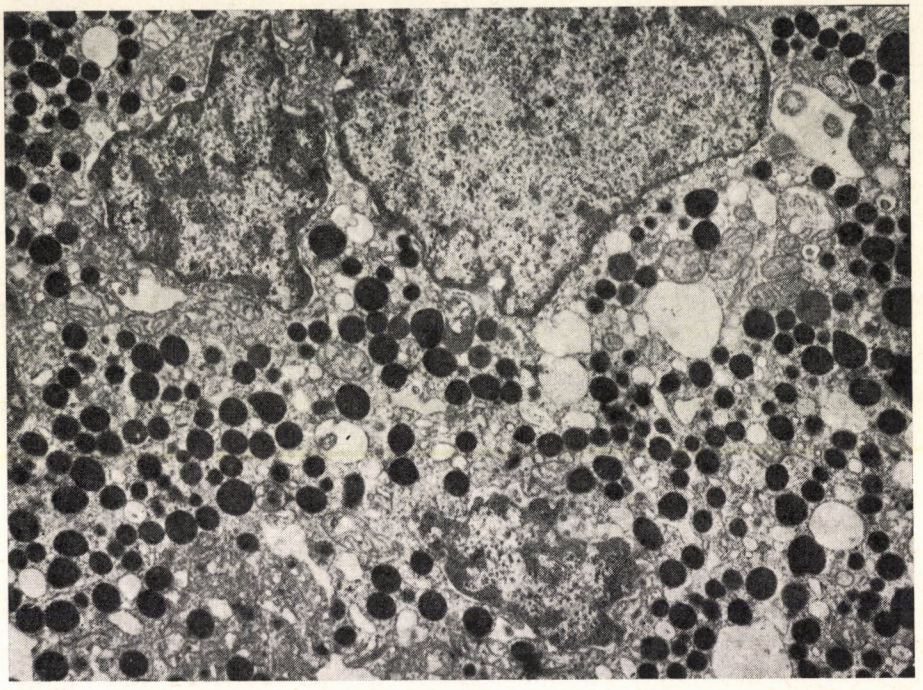


Fig. 6. Electron micrograph of the adenoma of patient 5: densely granulated prolactinoma. ×8000

turbances, for example in prolonged high-dosage oestrogen treatment of rats or in the course of chronic thyroid insufficiency. Pituitary hypertrophy or tumours have been noted in gonadal failure in experimental animals as well as in humans, and after total adrenalectomy a pituitary tumour has been seen to appear in Cushing's disease [1, 9, 15, 16, 26, 34, 35, 39, 42, 47, 49, 51]. One of our male patients (patient 1) had peripheral hypogonadism due to chromosomal aberration, while one female patient (patient 2) had multiple pregnancies and long-lasting primary hypothyroidism. Two other female (patients 7, 11) had taken contraceptive pills for several years. These factors may have played an important role in initiating the proliferative process.

Endocrine-active microadenomas do not necessarily produce lesser amounts of hormone than do macroadenomas. In the majority of microadenomas no relation exists between their size and the observed hormone level. Symptomatic microadenomas may develop into endocrine macroadenomas, while some remain stable over long periods and a few regress spontaneously. The majority of endocrine-active microadenomas reach the stage of macroadenoma. This growth may be stimulated by endocrine factors such as oestrogen administration or pregnancy in the case of prolactinomas, or adrenalectomy in the case of corticotropic adenomas [31]. In our experience no such

progression was observed during a follow-up period of 4 to 16 months. Pituitary adenomas usually grow expansively and only a few show an invasive tendency. The adenoma and the normal gland are usually sharply demarcated in acromegaly and not so sharply in prolactinomas [29].

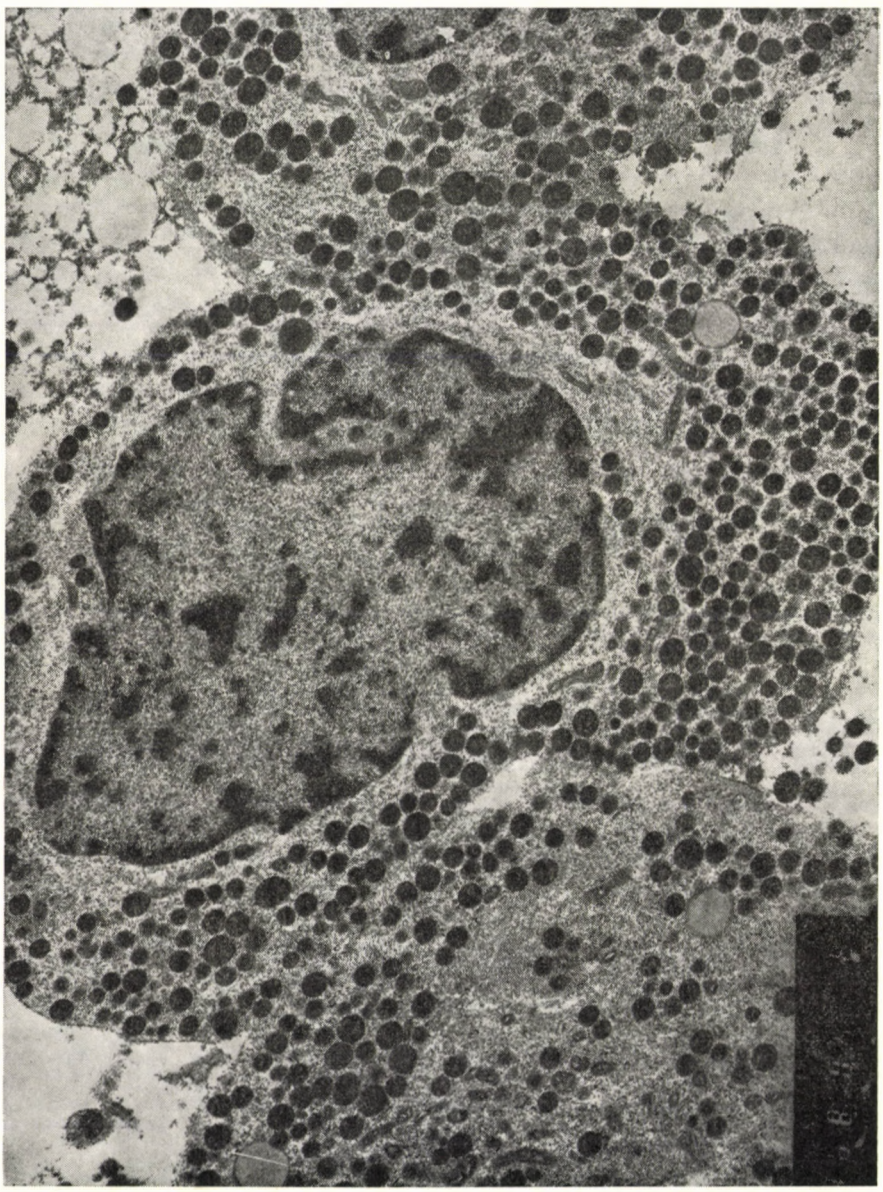


Fig. 7. Electron micrograph of the adenoma of patient 16: densely granulated GH cell adenoma. ×5000

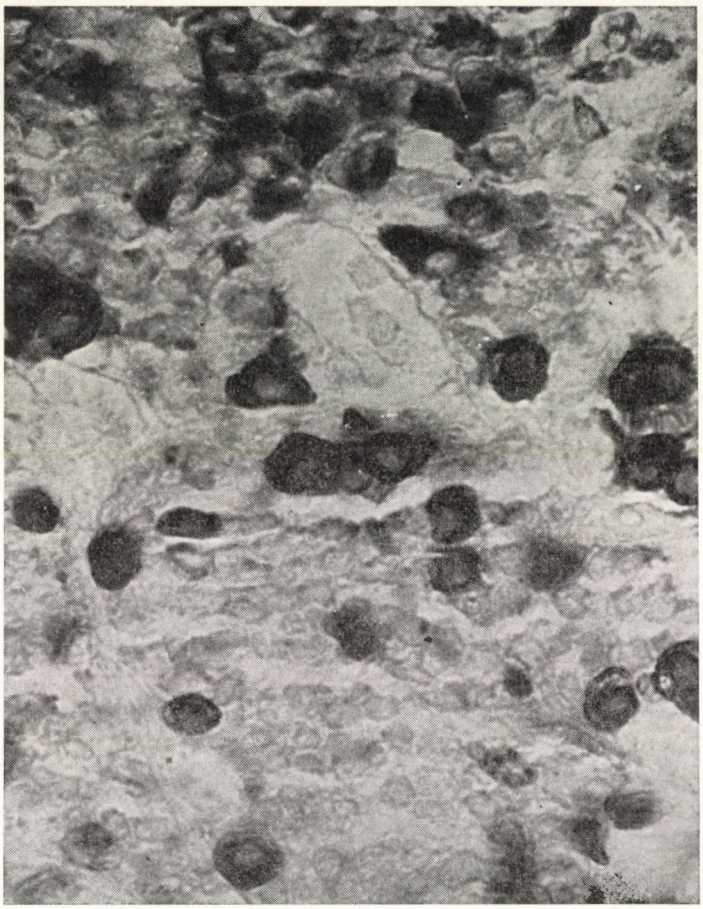


Fig. 8. Light micrograph of the adenoma of patient 3: immunoreactive PRL is evident in the cytoplasm. Immunoperoxidase technique

In contrast with the histologic techniques of Benda [3] and Erdheim [17] the introduction of immunocytology and electron microscopy allowed to divide the pituitary adenomas into well-defined entities [29].

The diagnosis of pituitary microadenoma is sometimes a complex task. The clinical symptoms (amenorrhoea, glactorrhoea, hypogonadism, infertility, impaired sexual performance, acromegaly, Cushing's and Nelson's syndromes) call for detailed radiological examinations. An enlarged pituitary fossa may be diagnosed from a plain skull radiograph. Angiograms have to be done when an aneurysm must be excluded and pneumo- or metrizamide encephalograms when there is a possibility of an "empty" sella [20, 41]. A CT scan will detect a microadenoma larger than 6–8 mm which reaches the entrance of the sella [4]. Frontal and sagittal thin-layer tomography of the sella is a use-

ful method for detecting small pituitary adenomas [52]. In all of our patients the sella was normal in size, but tomography then revealed its bulging, erosion or a sloping sign.

If the pituitary adenoma extends into the suprasellar region, compression of the optic pathways may occur with visual field defects. Microadenomas do not usually cause visual field defects or other symptoms of parasellar extension, but ophthalmic examination is of great importance in the follow-up of these patients.

Measurement of the anterior pituitary hormones is the other important method in the diagnosis of a functioning microadenoma. The elevated serum PRL level with the radiological signs mentioned above is usually a satisfactory proof of a pituitary adenoma. However, especially when a normal basal PRL level is accompanied by positive radiological and clinical symptoms, examination of the hormone reserve capacity is obligatory. Hypothalamic diseases and certain drugs, dopamine antagonists (neuroleptics, phenothiazines and metoclopramide) and catecholamine depletants (alpha-methyldopa and Rauwolfia alkaloids) may cause hyperprolactinaemia. When these factors have been excluded, 20–40% of the patients with hyperprolactinaemia show X-ray evidence of a pituitary adenoma [48]. Without any radiological suspicion of a microadenoma it is sometimes difficult to exclude the presence of a tumour. Recent investigations of PRL secretion have suggested that it is of questionable value to attempt to discriminate between microadenoma and dysfunctional hyperprolactinaemia [8, 27, 40, 46]. A raised basal hGH level which is not suppressed by oral glucose is diagnostic, but since hGH is released also during stress, a single basal hormone value is insufficient. In addition, derangement of carbohydrate tolerance can be determined by the oral glucose tolerance test. Inappropriate stimulation of the hGH level by TRH is found in 70%, and LH–RH in 30% of all acromegalic patients.

Two surgical approaches to the sella turcica exist. The trans-sphenoidal approach is used in nearly 90% of the patients, when the tumour is located within the sella or when there is a symmetrical extension into the suprasellar space. The transfrontal approach is selected for large presellar and laterally growing adenomas. In some patients, both trans-sphenoidal and transfrontal surgery must be done. Selective microadenomectomy is leading in most cases to a decrease of the hormone excess and the preservation of pituitary function [25, 45, 53]. In 50% of our operated prolactinoma patients, the serum PRL level decreased to the normal range while 60% of the acromegalic patients who underwent microadenomectomy showed a normal hGH level on glucose loading after the operation. The FSH, LH and TSH reserves were not significantly affected by the surgical intervention.

Irradiation treatment with heavy particles is possible in only a few centres in the world [32]. An alternative is conventional radiotherapy [33].

In this case, normalization of elevated hormone levels cannot be expected, and usually the hormone excess persists.

The implication of dopamine agonists in the treatment of hyperprolactinaemia and acromegaly has provided an alternative to surgery and irradiation [2, 11, 18]. For hyperprolactinaemic patients with a microadenoma but without evidence of suprasellar extension the drug of choice is bromocriptine. Bromocriptine was able to normalize the serum PRL level in 60% of our patients. Our suggested therapeutic scheme includes preoperative bromocriptine treatment for a 4–6 month period because of the beneficial effect of the drug on hormone production, and on the proliferation and demarcation of the tumour. A dramatic increase in size of the prolactinoma may sometimes occur during pregnancy. Primary bromocriptine treatment of hyperprolactinaemic women without examination of the sella is contraindicated. Bromocriptine is effective in some but not all cases of acromegaly, and some authors recommend an initial trial with the drug [23]. Doses of 10 to 60 mg per day are usually required. Normal levels of hGH can be restored in about 20% of the patients [18, 23]. In our experience low-dose bromocriptine treatment has a beneficial effect on the clinical symptoms of acromegaly [19], but this regimen achieved normalization of the serum hGH level in only 17% of our patients with acromegaly caused by pituitary microadenoma. A close correlation has not been found between the changes in the clinical symptoms and the decrease of the hGH level. In the cases of acromegaly caused by pituitary microadenoma, surgical treatment is preferable to bromocriptine.

Acknowledgements

The authors wish to thank Dr. Felicia Slowik and Dr. Katalin Bálint for electron microscopic and immunocytochemical examinations.

REFERENCES

1. Addison, W. H. F.: The cell changes in the hypophysis of the albino rats after castration. J. Comp. Neurol. **28,** 441–462 (1917).
2. Belforte, L., Camanni, F., Chiodini, P. G., Liuzzi, A., Massara, F., Molinatti, G. M., Müller, E. E., Silvestrini, F.: Long term treatment with 2-Br-α-ergocryptine in acromegaly. Acta Endocr. (Kbh.) **85,** 235–248 (1977).
3. Benda, C.: Beiträge zur normalen und pathologischen Histologie der menschlichen Hypophysis cerebri. Berl. Klin. Wschr., **36,** 1205–1210 (1900).
4. Bonneville, J.-F., Poulignot, D., Cattin, F., Couturier, M., Mollet, E., Dietemann, J.-L.: Computed tomographic demonstration of the effects of bromocriptine on pituitary microadenoma size. Radiology **143,** 451–455 (1982).
5. Camanni, F., Massara, F., Belfore, L., Molinatti, G. M.: Changes in plasma growth hormone levels in normal and acromegalic subjects following administration of 2-Br-ergocryptine. J. Clin. Endocr. **40,** 363–366 (1975).
6. Camanni, F., Massara, F., Belforte, L., Rosatello, A., Molinatti, G. M.: Effect of dopamine on plasma growth hormone and prolactine levels in normal and acromegalic subjects. J. Clin. Endocr. **44,** 465–473 (1977).
7. Camanni, F., Massara, F., Fassio, V., Molinatti, G. M., Müller, E. E.: Effect of five dop-

aminergic drugs on plasma growth hormone levels in acromegalic subjects. Neuroendocrinology **19,** 227–240 (1975).
8. Canales, E. S., Cano, C., Ablanedo, J., Zárate, A.: Nomifensine test does not differentiate hyperprolactinemia associated with and without radiologic abnormalities in the sella turcica. Hormone Res. **15,** 213–217 (1981).
9. Chiodini, P. G., Liuzzi, A., Botalla, L., Cremascoli, G., Silvestrini, F.: Inhibitory effect of dopaminergic stimulation on GH release in acromegaly. J. Clin. Endocr. **38,** 200–206 (1974).
10. Chiodini, P. G., Liuzzi, A., Müller, E. E., Botalla, G., Cremascoli, G., Opizzi, G., Verde, G. Silvestrini, F.: Inhibitory effect of an ergoline derivative methergoline on GH and PRL levels in acromegalic patients. J. Clin. Endocr. **43,** 356–363 (1976).
11. Clayton, R. N., Vrionides, Y., Lynch, S. S., Butt, W. R., London, D. R.: Response of acromegaly to long term bromocriptine therapy: a biochemical and clinical assessment. Acta Endocr. (Kbh.) **89,** 469–482 (1978).
12. Costello, R. J.: Subclinical adenoma of the pituitary gland. Amer. J. Path. **12,** 205–215 (1936).
13. Cushing, H.: The basophil adenomas of the pituitary body and their clinical manifestation (pituitary basophilism). Bull. Johns Hopkins Hosp. **50,** 137–195 (1932).
14. Delitala, G., Masala, A., Alagna, S., Devilla, L., Lotti, G.: Growth hormone and prolactin release in acromegalic patients following methergoline administration. J. Clin. Endocr. **43,** 1382–1386 (1976).
15. Dickie, M. M., Wolley, G. W.: Spontaneous basophilic tumors of the pituitary glands in gonadectomized mice. Cancer Res. **9,** 372–384 (1949).
16. Edwards, C. R. W., Forsyth, I. A., Besser, G. M.: Amenorrhoea, galactorrhoea and primary hypothyroidism with high circulating levels of prolactin. Brit. Med. J. **3,** 462–464 (1971).
17. Erdheim, J.: Zur normalen und pathologischen Histologie der Glandula thyreoidea, parathyroidea und Hypophysis. Beitr. Bath. Anat., **33,** 158–236 (1903).
18. Eskildsen, P. C., Svendsen, P. A., Vang, L., Nerup, J.: Long-term treatment of acromegaly with bromocriptine. Acta Endocr. (Kbh.) **87,** 687–700 (1978).
19. Gáspár, L., László, F. A.: Long-term bromocryptine treatment and somatostatin in acromegaly. Endokrinologie **76,** 152–162 (1980).
20. Gonsette, R. E.: Advances in ventriculography and myelography with the nonionic contrast medium, metrizamide. Acta Radiol. suppl. **347,** 467–475 (1975).
21. Guiot, G., Thibaut, B.: L'extirpation des adénomes hypophysaires par voie transsphénoidale. Neurochirurgia **1,** 133–139 (1959).
22. Hajda, M., Pásztor, E.: Significance of the appearance of the disc for predicting the visual functions following removal of pituitary adenomas. Neurol. Med.-Chir. (Tokyo) in press.
23. Halse, J., Haugen, H. N., Bohmer, T.: Bromocriptine treatment in acromegaly: clinical and biochemical effects. Acta Endocr. (Kbh.) **86,** 464–472 (1977).
24. Hardy, J.: Transsphenoidal microsurgery of the normal and pathological pituitary. Clin. Neurosurg. **16,** 185–190 (1969).
25. Hardy, J., Wigser, S.: Transsphenoidal surgery of pituitary fossa tumors with televised radiofluoroscopic control. J. Neurosurg. **23,** 612–615 (1965).
26. Jawadi, M. H., Ballonoff, L. B., Stears, J. C., Katz, F. H.: Primary hypothyroidism and pituitary enlargement. Arch. Intern. Med. **138,** 1555–1557 (1978).
27. Kamoi, K., Tchuchida, I., Sato H., Tanaka, R., Ishiguro, T., Kaneko, K., Iwasaki, Y., Shibata A.: Comparison of the responses in the nomifensine test with hyperprolactinemia due to prolactin-secreting pituitary tumors and nonprolactin-secreting hypothalamic tumors. J. Clin. Endocr. **53,** 1285–1287 (1981).
28. Kolodny, H. D., Sherman, L., Singh, A., Kim, S., Benjamin, F.: Acromegaly treated with chlorpromazine. New Engl. J. Med. **284,** 819–822 (1971).
29. Kovács, K., Horváth, E., Ezrin, C.: Pituitary adenomas. Path. Ann. **12,** 341–382 (1977).
30. Köbberling, J., Jüppner, H., Volkmann, B., Unger, K., Schwinn, G., Dirks, H.: Suppression of growth hormone by bromocryptine (CB 154) in acromegalics, lack of suppression in normals. Acta Endocr. (Kbh.) **78,** suppl. 193), 93 (1975).
31. Landolt, A. M.: Biology of pituitary microadenomas. In: Faglia, G. , Giovenelli, M., MacLeod, R. M. (eds): Pituitary Microadenomas. Academic Press, New York 1979, pp. 107–122.
32. Lawrence, J. H., Chong, C. Y., Lyman, J. T.: Treatment of pituitary tumors with heavy particles. In: Kohler, P. O., Ross, G. T. (eds): Diagnosis and Treatment of Pituitary Tumors. Excerpta Medica, Amsterdam 1973, pp. 253–262.
33. Lawrence, A. M., Pinsky, S. M., Goldfine, I. D.: Conventional radiation therapy in acromegaly: a review and reassessment. Arch. Intern. Med. **128,** 369–377 (1971).

34. Lawrence, A. M., Wilber, J. F., Hagen, T. C.: The pituitary and primary hypothyroidism: Enlargement and unusual growth hormone secretory responses. Arch. Intern. Med. **132,** 327–333 (1973).
35. Leiba, S., Landau, B., Ber, A.: Target gland insufficiency and pituitary tumours. Acta endocr. (Kbh.) **60,** 112–120 (1969).
36. Liuzzi, A., Chiodini, P. G., Botalla, L., Cremascoli, G., Müller, E. E., Silvestrini, F.: Decreased plasma growth hormone (GH) levels in acromegalics following CB-154 (2-Br-α-ergocryptine) administration. J. Clin. Endocr. **38,** 910–912 (1974).
37. Liuzzi, A., Chiodini, P. G., Botalla, L., Cremascoli, G., Silvestrini, F.: Inhibitory effect of l-dopa on GH release in acromegalic patients. J. Clin. Endocr. **35,** 941–945 (1972).
38. Malarkey, W. B., Johnson, J. C.: Pituitary tumors and hyperprolactinaemia. Arch. Intern. Med. 136, 40–46 (1976).
39. Moore, G. E., Brackney, E. El., Bock, F. G.: Production of pituitary tumors in mice by chronic administration of a thiouracil derivative. Proc. Soc. Exp. Biol. (N. Y.) **82,** 643–645 (1953).
40. Müller, E. E., Genazzani, A. R., Murru, S.: Nomifensine: diagnostic test in hyperprolactinemic states. J. Clin. Endocr. **47,** 1352–1357 (1978).
41. Neelon, F. A., Goree, J. R., Lebowitz, H. E.: The primary empty sella: Clinical and radiographic characteristics and endocrine function. Medicine **52,** 73–94 (1973).
42. Nelson, D. H., Meakin, J. W., Dealy, Jr., J. B., Matson, D. D., Emerson, Jr., K., Thorn, Y. W.: ACTH-producing tumor of the pituitary gland. New Engl. J. Med. **259,** 161–164 (1958).
43. Pásztor, E., Kemény, A. A., Piffkó, P.: Trans-sphenoidal surgery for suprasellar pituitary adenomas. Acta Neurochir. (Wien) in press. (1982).
44. Pásztor, E., Piffkó, P.: Trans-sphenoidal surgery and cerebrospinal surgery of cerebrospinal fluid rhinorrhoea. In: Rob, C., Smith, R. (eds): Operative Surgery. Neurosurgery. 3rd (ed.) Butterworths, London 1978, pp. 93–97.
45. Pásztor, E., Piffkó, P., Kemény, A., Hajda, M., Gábor, I.: Mai szemlélet a hypophysis adenomák sebészi kezelésére (Current views on the surgical treatment of hypophysis adenomas). Orv. Hetil. **122,** 1431–1438 (1981).
46. Pontiroli, A. E., Falsetti, L., Voltolini, A. M., Alberetto, M., Pellicciotta, G., de Pasqua., A., Girardi, A. M., Pozza, G.: Benserazide, a stimulator of prolactin release: a new test in the diagnosis of pituitary prolactin-secreting tumours. Acta Endocr. (Kbh.) **98,** 326–332 (1981).
47. Russfield, A. B.: Histology of the human hypophysis in thyroid disease-hypothyroidism, hyperthyroidism, and cancer. J. Clin. Endocr. **15,** 1393–1408 (1955).
48. Sherman, B. M., Schlechte, J.: Pathogenesis of prolactin-secreting pituitary adenomas. Lancet **2,** 1019–1021 (1978).
49. Stockigt, J. R., Essex, W. B., West, R. H.: Visual failure during replacement therapy in primary hypothyroidism with pituitary enlargement. J. Clin. Endocr. **43,** 1094–1100 (1976).
50. Tolis, G., Pinter, E. J., Friesen, H.: Metabolic and endocrine actions of 2-bromo-ergocryptine (CB-154). Int. J. Clin. Pharmacol. **10,** 151 (1974).
51. Van Wyk, J. J., Grumbach, M. M.: Syndrome of precocious menstruation and galactorrhea in juvenile hypothyroidism: An example of hormonal overlap in pituitary feedback. J. Pediatr. **57,** 416–435 (1960).
52. Vezina, J., Sutton, T. J.: Prolactin secreting pituitary microadenomas. Roentgenologic diagnosis. Amer. J. Roentgenol. **120,** 46–51 (1974).
53. Wilson, Ch. B.: Neurosurgical management of large and invasive pituitary disorders. In: Tindall G.T., Collins, W. F. (eds): Current Neurosurgical Management. Raven Press, New York 1979, pp. 395–442.

Acta Medica Hungarica 40 (2—3), pp. 125—132 (1983)

ADRENOCORTICAL FUNCTION IN BRONCHIAL ASTHMA

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(Received June 14, 1982)

Female patients with "intrinsic" bronchial asthma without corticosteroid therapy for at least 3 months revealed low blood concentration of total (protein-unbound + protein-bound) and free (biologically active, protein-unbound) cortisol, dehydroepiandrosterone and dehydroepiandrosterone sulphate, as also a low urinary excretion of dehydroepiandrosterone metabolites.

The results suggest that bronchial asthma is associated with hypadrenia due to an impaired production not only of cortisol, but also of adrenocortical androgens resulting in an insufficient hormone supply of the target organs. These seem noteworthy, since recent observations showed relationships between the immune responsiveness and sex hormones.

Keywords: Bronchial asthma, cortisol, dehydroepiandrosterone, free and protein-bound hormones, hypadrenia

Introduction

Pathologic processes involving abnormal immune reactions have long been known to respond favourably to corticosteroids. Bronchial asthma is a disease of this kind. The mechanism by which glucocorticoids exert their therapeutic effect is still unknown [2], and although corticoid metabolism in this disease has been widely studied, the results are unsatisfactory or conflicting [3, 11, 14]. On the evidence of our earlier studies, in "intrinsic" bronchial asthma both the basal serum cortisol level and the urinary excretion of this hormone decreased [7]. Further studies indicated that the adrenocortical responsiveness of patients with bronchial asthma to exogenous ACTH is inferior to that of normals, even in those without corticoid therapy [15].

In the present study the metabolic processes of cortisol and dehydroepiandrosterone, and their interaction with carrier proteins were examined. Since these are quantitatively the most important hormones in respect of the glandular production and their blood level, a comparative analysis of their function in bronchial asthma seemed justified.

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Patients and methods

Twenty healthy females with no endocrine abnormality aged from 21 to 65 years and 15 female patients aged from 18 to 65 years with "intrinsic" bronchial asthma were studied. The disease had a history of 3 to 30 years. The patients had been on steroids for 3 to 7 years, mostly in the form of maintenance therapy, occasionally as short-term, massive-dose courses. Before study, steroids were withdrawn for at least 3 months and bronchodilatators were given only. The patients were without endocrine, liver, renal disease and at the time of study they were free of symptoms.

The total (free and protein-bound) plasma cortisol levels ($TOTAL_F$) were measured by the spectrofluorimetric method of Mattingly [12] in blood samples taken at 8 a.m. For measurement a Hitachi Model 204 spectrofluorimeter was used. Since no separation technique was employed, the cortisol values included small amounts of other fluorogens.

Plasma total dehydroepiandrosterone ($TOTAL_D$) was measured by radioimmunoassay according to Abraham et al. [1].

The antiserum was raised against dehydroepiandrosterone-hemisuccinate-BSA in rabbits (BIODATA, England). An intraassay coefficient of variation was 9.1% and the interassay coefficient of variation 16.2%.

For the measurement of plasma total (bound + free) dehydroepiandrosterone sulphate ($TOTAL_{DS}$) and of androsterone sulphate ($TOTAL_{AS}$) our gas chromatographic method [9] was used.

For separation of "free" (protein-unbound) hormones equilibrium dialysis by means of radioactive steroids, after dilution of plasma to 1 : 5, according to the method of Westphal [17] was used. The specifically to globulin bound fraction ("binding globulin", BG) and the aspecifically to albumin bound (BA) fractions were separated by the thermoinactivation method of Daughaday [5]. For the radioactivity measurements Beckman Model LS 100 liquid-scintillation spectrometer was used. From the given total amount of the hormone (TOTAL) and the degree of protein-binding, the "actual" free (apparent free, AFHC) hormone concentration of the plasma could be calculated [5, 17].

Urinary excretion of the metabolites of the quantitatively most significant adrenocortical "androgenic" hormone, dehydroepiandrosterone, was determined by the method of Fehér [8] combining fractional hydrolysis, paper chromatography and spectrophotometry. These metabolites are:

dehydroepiandrosterone sulphate (DS),
androsterone sulphate (AS),
etiocholanolone sulphate (ES),
dehydroepiandrosterone glucuronate (DG),
androsterone glucuronate (AG),
etiocholanolone glucuronate (EG)

For the measurement of plasma total protein the biuret method of Weichselbaum, and for separation, paper electrophoresis was used.

The results were evaluated, if age had to be taken into consideration in two groups (18–45 years: functioning ovaries; 45–65 years; menopause). If no age-related differences were demonstrable, the results were evaluated in a single group. The control subjects were age-matched in each group. For statistical evaluation Student's two sample *t* test was used with the aid of a programmed Hewlett–Packard Model 97 calculator.

Results

Results are summed up in Tables I—IV, where the number of cases (n), the mean values ($\bar{x}$) with standard deviations (S.D.) and the results of statistical evaluation (P) have been given. The blood values comprise the total amount (free + bound: TOTAL), the specifically globulin-bound (e.g. transcortin, sex hormone binding globulin: BG) and the protein-unbound (i.e. the apparent free hormone concentrations: AFCH) fractions. From these figures the nonspecific hormone binding to albumin can be calculated.

Table I lists the results of plasma protein analysis. The plasma albumin concentration was significantly below the normal. Total globulin was normal, the α_1 and α_2 fractions were above the normal values. The albumin/globulin ratio was, therefore, below that of the controls, the differences being statistically significant.

Table I

Distribution of plasma protein fractions in bronchial asthma (mean ± S.D.)

Protein fractions	Normal values (n = 14)	Bronchial asthma (n = 6)	
Albumin			
Total value, g/l	41.0 ± 3.0	35.0 ± 5.0	$P < 0.0005$
Relative value	0.582 ± 0.020	0.556 ± 0.031	$P < 0.025$
Globulin			
Total value, g/l	30.0 ± 3.0	29.0 ± 2.0	
Fractions α_1	0.040 ± 0.000	0.048 ± 0.008	$P < 0.0025$
(relative values) α_2	0.080 ± 0.006	0.093 ± 0.021	$P < 0.025$
β	0.098 ± 0.013	0.107 ± 0.011	
γ	0.200 ± 0.021	0.197 ± 0.038	
Albumin/globulin	1.4 ± 0.1	1.2 ± 0.1	$P < 0.01$

n: number of cases

Table II

Distribution of plasma cortisol and dehydroepiandrosterone fractions (mean ± S.D.)

Unconjugated steroids in plasma	Normal values	Bronchial asthma	
Cortisol (F) fractions	n = 27	n = 10	
$TOTAL_F$, nmol/l	425.0 ± 113.2	218.0 ± 115.9	$P < 0.0005$
$AFHC_F$, nmol/l	52.4 ± 11.0	27.6 ± 8.3	$P < 0.0005$
$AFHC_F$, per cent	12.0 ± 4.2	12.6 ± 4.2	
BG_F, per cent	47.5 ± 4.3	48.7 ± 7.3	
Dehydroepiandrosterone (D) fractions	n = 25	n = 15	
$TOTAL_D$, nmol/l	20.7 ± 8.1	11.9 ± 9.8	$P < 0.005$
$AFHC_D$, nmol/l	2.1 ± 0.7	1.4 ± 0.4	$P < 0.01$
$AFHC_D$, per cent	10.1 ± 2.5	12.7 ± 4.0	$P < 0.01$
BG_D, per cent	6.9 ± 4.2	6.8 ± 4.2	

n: number of cases, TOTAL: protein-bound + free, AFHC: apparent free hormone concentration, BG: globulin-bound

In Table II the distribution of the cortisol (F) and dehydroepiandrosterone (D) fractions is shown. In bronchial asthma the $TOTAL_F$ was significantly below the normal. Though the percentual proportion of the BG_F and $AFCH_F$ values were normal, as a result of the low $TOTAL_F$, the absolute level of $AFCH_F$ was also below normal. Under basal conditions the blood cortisol level is thus abnormally low in bronchial asthma.

Unconjugated D was similar in quantity and distribution to F, both $TOTAL_D$ and $AFCH_D$ were below the normal values. Since in the case of this steroid no age-related differences were demonstrable, the results were evaluated together.

Table III shows the distribution of the blood DS and AS concentration in age groups of 18–45 and 45–65 years. This grouping was substantiated by the marked age-related decline of the DS value. In bronchial asthma the values for $TOTAL_{DS}$ and $TOTAL_{AS}$ were considerably lower than in the controls. Despite a fairly wide individual fluctuation of values, the differences were particulary marked in the age group of 18–45 years. It is noteworthy that the specific binding of DS to the globulin fractions, relative to that of F, was strikingly poor under both normal and pathological conditions (Tables II, III). On the other hand in bronchial asthma a higher proportion of the decreased $TOTAL_{DS}$ amounts occurred in the free ($AFHC_{DS}$) than in the bound

Table III

Distribution in plasma of dehydroepiandrosterone sulphate and androsterone sulphate (mean ± S.D.)

Conjugated steroids	Normal values	Bronchial asthma	
Dehydroepiandrosterone sulphate (DS) fractions:			
$Total_{DS}$, μmol/l	4.1 ± 1.5*	1.3 ± 1.0*	$P < 0.01$
	1.8 ± 0.7**	0.9 ± 0.6**	$P < 0.01$
$AFHC_{DS}$, μmol/l	0.26 ± 0.07	0.16 ± 0.07	
	0.11 ± 0.04	0.08 ± 0.03	
$AFHC_{DS}$, per cent	6.4 ± 1.8	12.0 ± 5.6	$P < 0.05$
	6.1 ± 1.9	8.6 ± 3.2	$P < 0.05$
BG_{DS}, per cent	1.8 ± 1.4	1.7 ± 1.8	
	4.4 ± 2.9	4.3 ± 1.5	
Androsterone sulphate (AS) fractions:			
$Total_{AS}$, μmol/l	1.4 ± 0.7*	0.5 ± 0.5*	$P < 0.0025$
	1.2 ± 0.5**	0.6 ± 0.5**	$P < 0.05$

* age 18–45 years (14 normal, 8 asthmatic)
** age over 45 years (8 normal, 8 asthmatic)
TOTAL: protein-bound + free, AFHC: apparent free hormone concentration, BG: globulin-bound

Table IV

Urinary excretion of dehydroepiandrosterone and its metabolites (mean ± S.D.)

Steroids in urine	Normal values	Bronchial asthma	
$Total_D$, μmol/24 h	4.2 ± 3.1*	0.4 ± 0.3*	$P < 0.01$
	1.1 ± 0.7**	0.1 ± 1.2	$P < 0.005$
$Total_{D+E+A}$ μmol/24 h	22.4 ± 8.8	4.2 ± 2.1	$P < 0.0005$
	12.6 ± 4.9	2.8 ± 2.1	$P < 0.0005$
DS, μmol/24 h	2.5 ± 2.4	0.3 ± 0.1	$P < 0.05$
	0.7 ± 1.0	0.1 ± 0.2	
DS + ES + AS, μmol/24 h	4.2 ± 3.9	1.1 ± 0.7	$P < 0.05$
	1.1 ± 1.4	0.4 ± 0.7	
DG, μmol/24 h	0.4 ± 0.4	0.1 ± 0.1	$P < 0.05$
DG + EG + AG, μmol/24 h	9.5 ± 3.9	4.6 ± 2.8	$P < 0.02$
	5.3 ± 4.2	1.8 ± 2.5	$P < 0.05$

* aged 18–45 (12 normal, 6 asthmatic)
** aged over 45 (11 normal, 9 asthmatic)
TOTAL: free + sulphate + glucuronate, D: dehydroepiandrosterone, E: etiocholanolone, A: androsterone, S: sulphate, G: glucuronate

(BG_{DS}) form, nevertheless as a result of the marked $TOTAL_{DS}$ deficit, the absolute amounts of the free hormone were below the normal range. Thus not only cortisol but also the other quantitatively most significant hormone, dehydroepiandrosterone, had an abnormally low blood level in bronchial asthma.

Table IV shows the urinary excretion of total dehydroepiandrosterone and its metabolites (non-esterified + sulphate + glucuronate) and of the simultaneously measured sulphate and glucuronate fractions. In spite of wide individual variations, it is clearly seen that in bronchial asthma renal elimination of metabolites of adrenocortical "androgens" is considerably lower than in the control group. The decrease was significant for almost every metabolite.

The finding of a reduced metabolite excretion has lent support to our earlier supposition that the cortisol and sex hormone deficiency in the blood of patients with bronchial asthma results from an impairment of glandular hormone production.

Discussion

Bronchial asthma is a disease caused by a variety of factors. The role of corticosteroids in the management of some of its types and the involvement of certain molecular processes of the neuroendocrine system in its pathomechanism are well known [3, 10, 11, 14, 15].

In order to gain further information on the steroid metabolism in this complex disease we extended our study in addition to cortisol to adrenocortical sex hormones.

Immune reactions are to play an essential role in many cases of bronchial asthma. There is, on the other hand, mounting evidence that the sex hormones may affect the immune reactions and influence autoimmune processes at the molecular level [4, 16]. The male sex hormones have a suppressive effect on antibody production which is believed to account for the sex differences in the immune responses [4].

These considerations prompted us to study the metabolic processes of the other main adrenal steroid, dehydroepiandrosterone. This seemed the more justified since no pertaining data for this androgen have been found in the literature.

Our observation that patients with bronchial asthma under long-term treatment with corticoids have an abnormally low blood cortisol level ($TOTAL_F$) is consistent with the findings of other authors. Low cortisol level has been demonstrated also in patients who had never received corticoids [6, 10, 13], a finding suggestive of some degree of hypadrenia in bronchial asthma [10, 13]. We have been able to demonstrate that the impaired adrenocortical function in this disease involves the adrenocortical "androgens" as well, since the blood levels of $TOTAL_D$, $TOTAL_{DS}$, $TOTAL_{AS}$, and the urinary excretion of the metabolites of these hormones were found below the normal values.

It is generally accepted that the protein-unbound (unconjugated) hormone fraction in the blood comprises the biological activity and the availability of a hormone to the subcellular receptors, in many case independent of the production rate. Therefore the apparent free hormone concentration (AFHC) has a value to assess the hormone supply. In the present study we have estimated the $AFHC_F$, $AFHC_D$ and $AFHC_{DS}$ levels as also their reversibly inactivated (specifically to carrier protein bound) fractions, the BG_F, BG_D and BG_{DS} levels. From these hormone fractions those bound nonspecifically to albumin can also be calculated.

According to our observation in bronchial asthma the plasma protein spectrum differs from the normal pattern, the albumin level being below, and that of the α_1 and α_2 globulin fractions above the normal value.

In the cases of bronchial asthma not only the total (TOTAL) hormones but also those circulating in an active form ($AFHC_F$, $AFHC_D$ and $AFHC_{DS}$) failed to attain the values of the normal controls, which points to an inadequate hormone supply of the target organs.

It is further point of interest that the percentual proportion of $AFHC_D$ and of $AFHC_{DS}$ thus were significantly above the normal range, which would seem to indicate that in bronchial asthma the protein spectrum is shifted towards a proportional increase of active (i.e. protein-unbound) D and DS.

However this shift seems not to be operative in the case of F, and appears to be insufficient for adrenocortical androgens, presumably as a result of decreased glandular hormone production.

The present findings suggest that the endocrine defect in bronchial asthma is not confined to corticoid metabolism, but also affects certain metabolic processes of the sex hormones. The relationships between the sex hormones and the immune system indicated by recent studies [4, 16], make it justified to persue the studies of the endocrine system in the bronchial asthma.

It has to be mentioned that in preliminary trials additional dehydroepiandrosterone therapy seemed to be beneficial, it allowed to reduce the doses and thus to attenuate the side-effects in patients who responded only to massive-dose corticoid therapy.

REFERENCES

1. Abraham, G. E., Buster, J. E., Kyle, F. W., Carales, P. C., Teller, R. C.: Radioimmunoassay of plasma pregnenolone, 17-hydroxypregnenolone and dehydroepiandrosterone under various physiological conditions. J. Clin. Endocr. Metab. **37,** 140–144 (1973).
2. Aviado, D. M., Carrillo, L. R.: Antiasthmatic action of cortocosteroids: A review of the literature on their mechanism of action. J. Clin. Pharmacol. **10,** 3–11 (1970).
3. Blumenthal, M. N., McLean, J. A., Matthews, K. P.: Adrenal-pituitary function in bronchial asthma. Arch. Intern. Med. **117,** 34–38 (1966).
4. Cohn, D. A.: High sensitivity to androgen as a contributing factor in sex differences in the immune response. Arthr. and Rheum. **22,** 1218–1233 (1979).
5. Daughaday, N. H.: Binding of corticosteroids by plasma protein. III. The binding of corticosteroids and related hormones by human plasma and plasma protein fractions as measured by equilibrium dialysis. J. Clin. Invest. **37,** 511–519 (1958).
6. Dwyer, J., Lazarus, L., Hickie, J. B.: A study of cortisol metabolism in patients with chronic asthma. Aust. Ann. Med. **16,** 297–304 (1967).
7. Fehér, G. K., Koó, É.: Mellékvesekéreg hormon vizsgálatok asthma bronchiales betegeken. Meeting of Hungarian Allergological and Immunological Association. Kékestető 1977.
8. Fehér, T.: Determination of individual unconjugated 17-ketosteroids and 17-ketosteroid sulphates and glucuronosides in human urine. Acta Med. Acad. Sci. hung. **28.** 227–238 (1971).
9. Fehér, T., Bodrogi, L., Fehér, G. K., Poteczin, E.: Gas-chromatographic determination of free and solvolysable dehydroepiandrosterone and androsterone in blood. Chromatographia **10,** 86–91 (1977).
10. Jakobson, T., Sundberg, M., Kotovirta, M. L., Strandström, L.: Pituitary-adrenal function in patients given long-term adrenal steroid therapy. Acta Endocr. (Kbh.) **59,** 76–88 (1968).
11. Kilborn, J. R., Robson, A. O.: Studies of adrenocortical function in continuous asthma. Thorax **20,** 93–98 (1965).
12. Mattingly, D.: A simple fluorometric method for the estimation of free 11-OH-corticoids in human plasma. J. Clin. Path. **15,** 374–379 (1962).
13. Seigel, S. C., Heimlich, E. M., Richards, W., Kelley, V. C.: Adrenal function in allergy: effect of dexamethasone aerosols in asthmatic children. Pediatrics **33,** 245–251 (1964).
14. Shapiro, G. G., Tottoni, D. S., Pierson, W. E., Kelley, V. C., Bierman, V. W.: Physiology of steroid dependent and non-steroid dependent asthmatic youths. Ann. Allergy **38,** 237–241 (1977).
15. Sundberg, M., Jakobson, T.: Comparison of pituitary-adrenal function in asthmatic patients given long term corticosteroid therapy and in asthamtics who have not received adrenal steroids. Acta Allerg. (Kbh.) **24,** 11–26 (1969).
16. Steinberg, A. D., Melez, K. A., Raveche, E. S., Reeves, J. P., Boegel, W. A., Smathers, P. A., Taurog, J. D., Weinlein, L., Duvic, M.: Approach to the study of role of sex hormones in autoimmunity. Arthr. and Rheum. **22,** 1170–1176 (1979).
17. Westphal, U.: Corticosteroid binding globulin and other steroid hormone carriers in the blood stream. J. Reprod. Fertil. Suppl. **10,** 15–38 (1970).

Acta Medica Hungarica 40 (2—3), pp. 133—138 (1983)

SERUM LIPIDS DURING STARVATION IN OBESITY

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(Received September 10, 1982)

The HDL-cholesterol level was found to decrease during the first week of therapeutic starvation in hyperlipoproteinaemic (hypertriglyceridaemic), diabetic (non-insulin dependent) patients. The possible causes of the finding are discussed, and the view is expressed that the fall in HDL gives no cause for discontinuing the caloric restriction or starvation as the therapeutic measures in obesity.

Keywords: HDL-cholesterol, hypertriglyceridaemia, obesity, non-insulin dependent diabetes mellitus, total energy restriction

Introduction

Total starvation for rapid loss of weight has been resorted to for years in the management of obesity, particularly in non-insulin dependent diabetes mellitus (NIDDM) in the interest of metabolic control. A fall in the total cholesterol (CHOL) and triglyceride (TG) levels has been demonstrated in earlier studies [6, 8]. The relationship between the CHOL concentration of certain lipoprotein (LP) fractions with ischaemic heart disease and with other processes of atherosclerotic origin have become known in recent years. While high-density lipoprotein (HDL) CHOL is claimed to display a protective effect against atherosclerosis, an atherogenous effect is attributed to low-density lipoprotein (LDL) CHOL [4, 10]. It seemed therefore justified to study the changes in the serum CHOL and TG levels and the LP fractions in starved patients, in order to find out whether starvation causes an atherogenous shift in the LP.

Patients and methods

Nine patients were studied, 4 males of 51 (42–56) years and 5 females of 36.2 (33–42) years age average. Ideal body weight was calculated on the basis of Broca's formula. Overweight before starvation averaged 33.2% (23.3–43.4%) for the males and 59% (23.5–121.4%) for the females. Seven of the 9 patients were hyperlipoproteinaemic; the classification of hyperlipoproteinaemia (HLP) was based on the criteria recommended by WHO [3]. HLP was of

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Table I

Data of patients

Serial No.	Sex	Age	Diagnosis	HLP-type	Height cm	Weight kg	Overweight kg	Duration of starvation weeks	Weight loss kg
1	M	56	NIDDM Obesity and HLP	IV	187	112	+25	1	—5
2	M	52	NIDDM Obesity and HLP	IV	174	106	+32	2	—6
3	M	54	NIDDM Obesity and HLP	IV	160	74	+14	1	—3
4	F	36	Obesity	—	165	112	+47	3	—9
5	F	42	Obesity and HLP	IV	156	124	+68	2	—9
6	M	42	NIDDM Obesity and HLP	II/B	161	85	+24	1	—4
7	F	37	NIDDM Obesity and HLP	IV	155	83	+28	2	—9
8	F	33	NIDDM Obesity	—	168	84	+16	1	—4
9	F	33	Obesity and HLP	IV	174	94	+20	3	—12

type II/B in one case, and of type IV in the other 6 cases. Six patients had NIDDM, in one of them HLP of type II/B, in four HLP of type IV was present. The duration of complete starvation was 1 week in 4 cases, 2 weeks in 3 cases and 3 weeks in 2 cases. The data of the patients are shown in Table I.

The CHOL level in the sera and the LP fractions were measured by an enzymatic method (CHOD-PAP Boehringer), the TG concentration by the method of Grafnetter [5]. For LP electrophoresis agarose was used. CHOL and TG in the LP classes were estimated as follows: very-low-density (VLD) and low-density (LD) LP were precipitated in polyethylene glycol [1], centrifuged and the HDL-CHOL concentrations were measured in the supernatant. The LDL + HDL-CHOL and TG concentrations were estimated according to Wilson and Spiger [14]: after precipitation of the VLDL and centrifuging CHOL and TG were measured in the subnatant. Subtraction of the known HDL-CHOL value from LDL + HDL-CHOL gave the concentration of LDL-CHOL. From these VLDL-CHOL = serum CHOL-(LDL + + HDL-CHOL). The VLDL-TG concentrations were calculated by subtraction of LDL-TG + + HDL-TG from the total TG. For analysis of the results the one sample *t* test was used.

Results

Weight loss of the patients during starvation was in conformity with published results, including our earlier observations [2]. Mean weight loss during the first week was 4.6 (3–6 kg). The patients continuing on this therapy lost another 4 kg during the second, and 2 kg during the third week.

The serum lipids were checked weekly. The data for serum total CHOL and, within this for LDL, VLDL and HDL-CHOL, are shown in Table II. Table III shows the data relating to HDL-CHOL in detail.

The fall of the CHOL level amounted to 7.6% at the end of first and to 13.1% during the second week. The individual classes of CHOL showed

the following changes. VLDL-CHOL declined continously at the highest rate, LDL-CHOL increased slightly during the first week and decreased from the second week onward. HDL-CHOL declined during the first week, but remained unchanged from the second week onward. The LDL-CHOL/

Table II

Serum cholesterol level during starvation (mean ± S.D.)

Week	n	Total-CHOL mmol/l	VLDL-CHOL mmol/l	LDL-CHOL mmol/l	HDL-CHOL mmol/l	LDL-CHOL mmol/l / HDL-CHOL mmol/l
0	(9)	5.48 ± 1.13	1.18 ± 0.47	2.91 ± 0.82	1.39 ± 0.26	2.15 ± 0.72
1	(9)	5.16 ± 0.86	0.94 ± 0.44	2.96 ± 0.77	1.25 ± 0.46	2.65 ± 1.13 $P < 0.05$
2	(5)	4.36 ± 0.90	0.69 ± 0.15	2.43 ± 0.62	1.24 ± 0.34	1.97 ± 0.53

Table III

Response of HDL-CHOL (mmol/l) to starvation

Serial No.	Week 0	Week 1	Week 2
1	1.57	2.12	—
2	1.10	1.21	0.97
3	1.20	1.08	—
4	1.31	1.21	1.28
5	1.12	0.82	1.20
6	1.20	0.90	—
7	1.41	0.98	0.97
8	1.44	0.98	—
9	2.19	1.97	1.80
mean ± S.D.	1.39 ± 0.26	1.25 ± 0.46	1.24 ± 0.34

Table IV

Serum triglyceride concentrations (mmol/l) during starvation (mean ± S.D.)

Week	n	Total TG	VLDL-TG	(LDL-TG) + + (HDL-TG)
0	(9)	3.46 ± 1.95	1.53 ± 1.29	1.94 ± 0.92
1	(9)	2.50 ± 1.35*	1.25 ± 0.91	1.30 ± 0.51*
2	(5)	1.77 ± 0.61*	0.87 ± 0.39	0.90 ± 0.32*

* referred to the initial value (0) $P < 0.05$

Table V

CHOL and TG concentrations of VLDL during starvation (mean)

Week	n	VLDL-TG	VLDL-CHOL mmol/l	VLDL $\frac{TG}{CHOL}$
0	(9)	1.53	1.18	1.29
1	(9)	1.25	0.94	1.33
2	(5)	0.87	0.69	1.28

HDL-CHOL quotient increased significantly during the first week, but fell below the initial values during the second week.

During starvation a continuous fall in total TG was noted. Within this the decline of TG on the LP classes was slightly more marked than of those of VLD (Table IV).

Our studies of VLDL, which are delivered by the liver into the circulation, provided information on the proportion of decline of both VLDL-CHOL and TG. Calculation of the quotients of the mean VLDL-TG/VLDL-CHOL concentrations showed that the proportion of CHOL and TG within the declining concentrations of VLDL remained practically stable (Table V).

Discussion

In agreement with earlier findings, a decrease in serum total CHOL and TG was noted during starvation therapy of our prevalently hyperlipaemic obese patients with NIDDM. The changes in the individual LP classes in the course of starvation were, however, divergent. In the VLDL, released for the greatest part by the liver into the circulation, a proportional decline of the CHOL and TG concentrations was found. The equal fall in the CHOL and TG concentrations may be attributed to a diminished production of total VLDL. Direct proof of this has been furnished by Mancini et al. [9] by determination of the protein moiety of the VLDL molecule.

During the first week of starvation, the obese patients displayed a decrease in HDL-CHOL and minor increase in LDL-CHOL, parallel with a decrease in the total CHOL level. Changes in LDL and HDL-CHOL of a similar direction have been reported by Mancini et al. [9] in the course of starvation of prevalently normolipaemic obese patients, with the difference that during the first week the total CHOL concentration was also found to increase. A decrease in HDL-CHOL was noted by Jungmann et al. [7] during the first week of starvation, and by Taskinen and Nikkilä [12] in the case of a diet supplying 400 cal/day. Thompson et al. [13] studying the joint effect

of low-energy intake and increased physical exercise in non-diabetic and non-HLP obese subjects, also found a decrease in HDL-CHOL, parallel with the loss of weight. Considering the formation and catabolism of HDL, it is well within the possibilities that in the first week of starvation either the rate of conversion of VLDL to LDL is increased, or the biological half-life of LDL is prolonged. For the second alternative possible modifications of the binding properties of the LDL-receptors, or a depression of the intracellular metabolism of LDL might provide a basis.

Taskinen and Nikkilä, examining the response of the LDL-CHOL/HDL-CHOL ratio to caloric restriction in normal subjects, as well as in obese normolipaemic and hypertriglyceridaemic patients being on 400 calories/day, attribute the reduction of HDL-CHOL to a depression of lipoprotein lipase activity in skeletal muscle [11, 12], in view of the fact that the conversion of TG-rich LP to HDL requires a normal LP lipase activity. A depression of intestinal apoLPA-I and A-II synthesis under the effect of starvation has also to be taken into account.

A fall in the HDL-CHOL level has been noted in the present study during the first week of starvation, prevalently in patients with NIDDM and HL. In our view this finding may be interpreted as a transitory sign of metabolic adaptation to starvation. Even in consideration of the possible role of HDL-CHOL, the biochemical alterations resulting from their decline provide, in our view, no justification for discontinuing the caloric restriction or starvation in the management of obesity.

REFERENCES

1. Allen, J. K., Hensley, W. J., Nicholis, A. V., Whitfield, J. B.: An enzyme and centrifugal method for estimating high-density lipoprotein cholesterol. Clin. Chem. **25,** 325–327 (1979).
2. Balázsi, I., Stützel, M., Varsányi-Nagy M., Littmann, L., Szigeti, Á., Láng, I.: Éhezés hatása elhízottak glucose tolerantiájára, plasma insulin és serum szabad zsírsav szintjére. (Effect of fasting on the glucose tolerance, plasma insulin and serum free fatty acid levels in obese patients). Orv. Hetil. **115,** 249–253 (1974).
3. Beaumont, J. L., Carlson, L. A., Cooper, G. R., Fejfar, Z., Fredrickson, D. S., Strasser, T.: Classification of hyperlipidaemias and hyperlipoproteinaemias. Bull. Wld Hlth Org. **43,** 891–915 (1970).
4. Gordon, T., Castelli, W. P., Hjortland, M. C., Kannel, W. B., Dawber, T. R.: High density lipoprotein as a protective factor against coronary heart disease. The Framingham study. Amer. J. Med. **62,** 707–714 (1977).
5. Grafnetter, D.: Zjednoduzené stanoveni triglyceridu pro diagnostiku hyperlipidemii. Vnitrni Lék. **19,** 89–92 (1973).
6. Jackson, I. M. D.: Effect of prolonged starvation on blood lipid levels of obese subjects. Metabolism **18,** 13–17 (1969).
7. Jungmann, H., Stiehm, M., Harm, K.: Einfluss von Nahrungskarenz und Bewegungstherapie auf die Blutfette und ihre Fraktionen. Klin. Wschr. **59,** 1061–1064 (1981).
8. Mancini, M., Portiglione, A., DiMarino, L.: Feedback regulation of metabolism by dietary constituents: Lipids. Nutr. Metab. **21,** 13–25 (1977).
9. Mancini, M., Strazzullo, P., Contaldo, F., Postiglione, A., Riccardi, G., Perotti, N., Iovine, C.: Effect of prolonged fasting on plasma lipoprotein composition in obese patients.

In: Hessel, L. W., Krans, H. M. J. (eds): Lipoprotein Metabolism and Endocrine Regulation. North Holland Biomedical Press, Amsterdam 1979, pp. 35–43.
10. Miller, G. J., Miller, N. E.: Plasma high-density-lipoprotein concentration and development of ischaemic heart disease. Lancet **1,** 16–19 (1975).
11. Taskinen, M. R., Nikkilä, E. A.: Effects of caloric restriction on lipid metabolism in man. Atherosclerosis **32,** 289–299 (1979).
12. Taskinen, M. R., Nikkilä, E. A.: Lipoprotein lipase of adipose tissue and skeletal muscle in human obesity: response to glucose and to semistarvation. Metabolism **30,** 810–817 (1981).
13. Thompson, P. D., Jeffery, R. W., Wing, R. R.: Unexpected decrease in plasma high-density lipoprotein cholesterol with weight loss. Amer. J. Clin. Nutr. **32,** 2016–2021 (1979).
14. Wilson, D. E., Spiger, M. J.: A dual precipitation method for quantitative plasma lipoprotein measurement without ultracentrifugation. J. Lab. Clin. Med. **82,** 473–482 (1973).

Acta Medica Hungarica 40 (2—3), pp. 139—145 (1983)

STEROIDS EXCRETED BY HUMAN SKIN
I. C_{19}-steroids in axillary hair

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(Received October 26, 1982)

Seven free androgen steroids and four C_{19}-steroid sulphates were isolated and determined from the axillary hair of sexually mature healthy males and females. In 1 g axillary hair the free 5-androstene-3β, 17β-diol, testosterone, 5α-dihydrotestosterone, dehydroepiandrosterone, androsterone, 4-androstene-3,17-dione and 5α-androstane--3,17-dione occurred in nanomol amounts, whereas dehydroepiandrosterone sulphate, androsterone sulphate and 5-androstene-3β,17β-diol-3-sulphate were found in approximately 1000 times these amounts. The quantity of testosterone sulphate lies among the values for the free steroids. The free steroids present in hair are regarded as products of the sebaceous glands, and the sulphate ester steroids as products of the sweat glands.

Keywords: axillary hair, C_{19}-steroids, human skin

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-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-5α-androstan-17-one, Testosterone sulphate (Test-S) = 17β-sulphooxy-4-androsten-3-one

Introduction

Investigations during the past 15 years have revealed that the most important biochemical processes in androgen metabolism take place in the human skin [1–5]. In spite of this, few data are available on the steroids excreted by the skin [6, 7]. The present studies were centred on the hair where the substances excreted by the skin are enriched [7].

It was shown earlier that axillary hair, the type of hair richest in steroids, contains an appreciable quantity of 17-ketosteroid sulphates [7]. Of these, the main component is dehydroepiandrosterone sulphate (DHA-S), with a smaller amount of androsterone sulphate (And-S). In addition to these water-soluble steroid sulphates, certain free 17-ketosteroids (17-Ks) were isolated

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and identified from lipids coating the axillary hair [7]: 5α-androstane-3,17--dione (A-ane-dione), 4-androstene-3,17-dione(Δ^4-dione), dehydroepiandrosterone (DHA) and androsterone (And).

An account is now presented of the quantitative determination of C_{19}-steroids in axillary hair by colour reactions and protein-binding assay (PBA).

Materials and methods

1. The chemicals and solutions employed were of analytical purity. Organic solvents were purified and then distilled in a fractionating column [7].

2. Radioactive steroids. The following labelled steroids, products of the Radiochemical Centre (Amersham, England) or the New England Nuclear (USA), were used:

[1β,2β(n)-^{3}H] testosterone (S.A.: 44.6 Ci/mmol);
[1,2(n)-^{3}H] dihydrotestosterone (S.A.: 44 Ci/mmol);
[1,2-^{3}H(N)] androsterone (S.A.: 40 Ci/mmol);
[7-^{3}H(N)] dehydroepiandrosterone sulphate, ammonium salt (S.A.: 24 Ci/mmol);
[7-^{3}H(N)] 5-androstene-3β,17β-diol (S.A.: 24 Ci/mmol);

Before use, these steroids were purified by column and thin-layer chromatography [7]. [1β,2β(n)-^{3}H] 4-androstene-3,17-dione (S.A.: 44,6 Ci/mmol) was prepared from [1β,2β(n)-^{3}H] testosterone, and [1,2-^{3}H(N)]5α-androstane-3,17-dione (S.A.: 40 Ci/mmol) from [1,2-^{3}H(N)]-androsterone by Cr_2O_3 oxidation, while [7-^{3}H(N)]5-androstene-3β,17β-diol-3-sulphate (S.A.: 24 Ci/mmol) was prepared from [7-^{3}H(N)]dehydroepiandrosterone sulphate ammonium salt by $NaBH_4$ reduction. These steroids were purified on an Al_2O_3 column (Brockmann III/IV activity) and by thin-layer chromatography [7].

3. Radioactivity was measured with a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3255), 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 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 a florisil column (Florisil 60/100 mesh, Floridin Co., USA) [8]. The free steroids of different polarities were separated on an Al_2O_3 column with benzene containing an increasing ethanol concentration gradient [7]. The free steroids with similar polarities were separated on Al_2O_3-G (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) [7].

6. Chemical reactions of the C_{19}-steroids

a. The C_3- and C_{17}-keto groups of the free 17-ketosteroids in axillary hair were reduced to hydroxy groups with a methanolic solution of $NaBH_4$ under suitable experimental conditions:

4-androstene-3,17-dione → testosterone;
5α-androstane-3,17-dione → 5α-androstane-3α,17β-diol;
androsterone → 5α-androstane-3α,17β-diol;
dehydroepiandrosterone → 5-androstene-3β,17β-diol;
dehydroepiandrosterone sulphate → 5-androstene-3β,17β-diol-3-sulphate

The procedures were detailed previously [7].

b. Solvolysis of the C_{19}-steroid sulphates, acetylation of the free steroids, alkaline hydrolysis of the steroid acetates and oxidation of the hydroxy groups were carried out as described earlier [7].

7. Axillary hair specimens were obtained from healthy male and female laboratory workers, whose clothing corresponded to the normal Central European climate. Previous washing was done with soap and water. Before analysis, the plucked axillary hairs were weighed in the air-dry state [7].

8. Isolation and determination of C_{19}-steroids from axillary hair: The 200–400 mg of axillary hair was steeped in a mixture of 2 N NH_4OH and diethyl ether (1 : 1) to extract the free and sulphate ester steroids without decomposition [7]. The ether phase contained the free steroids and the aqueous phase the steroid sulphates. The labelled steroids of high specific activity were then added to both crude extracts to correct the losses occurring during

isolation. The crude extracts were first chromatographed on florisil columns. The free steroid fraction and the free steroids obtained after solvolysis of the steroid sulphates were separated into steroid groups on Al_2O_3 columns. The individual steroids were subsequently isolated on Al_2O_3-G thin layer.

The DHA-S and And-S isolated in larger amounts were determined with the Zimmermann and dinitrophenyl-hydrazine colour reactions [9], and the other steroids by PBA [10, 11]. The results were expressed in μmol or nmol per g axillary hair.

Results

Table I presents data on the identification and measurement of steroid sulphates isolated after consecutive column and thin-layer chromatographic purification and separation. DHA-S and And-S were found in the axillary hair in such large amounts that following isolation and solvolysis they could be determined by the Zimmermann and dinitrophenylhydrazine colour reactions. The quantities of 17-ketosteroid found by means of these two colour reactions agreed well, and thus the final result was reckoned from the values given by the two different procedures.

Two separate aliquots were subjected to PBA to determine the amount of Δ^5-diol-S (after solvolysis and subsequent thin-layer chromatography) as free Δ^5-diol. The residual steroid was acetylated, and the acetylated derivative was again run in system $1a_0$; the Δ^5-diol-diacetate dissolved out of the layer

Table I

Identification and measurement of C_{19}-steroid sulphates isolated from axillary hair following solvolysis

Isolated steroids	Chemical reaction	Derivatives	TLC systems	Methods used	Corrected according to radioactivity and calculated for 1 g of hair	
					values	mean
DHA-S	—	free steroid	G	Zimmermann-r	4.76 μmol/g	4.63 μmol/g
	2,4-DNP	DHA-2,4-DNPH	6	DHA-2,4-DNPH	4.51 μmol/g	
And-S	—	free steroid	G	Zimmermann-r	0.43 μmol/g	
	2,4-DNP	And-2,4-DNPH	6	And-2,4-DNPH	0.44 μmol/g	0.44 μmol/g
Δ^5-diol-S	—	free-steroid	G	PBA	4.80 μmol/g	
	Acetylation	Δ^5-diol-diAc	$1a_0$	PBA	3.42 μmol/g	
	Hydrolysis	Δ^5-diol	4	PBA	3.88 μmol/g	4.03 μmol/g
Test-S	—	free steroid	G	PBA	1.54 nmol/g	
				PBA	1.60 nmol/g	
	Oxidation	Δ^4-dione	G	PBA	1.16 nmol/g	
	Reduction	Test	G	PBA	1.10 nmol/g	1.35 nmol/g

TLC systems: G = Al_2O_3-G, n-hexane — ethyl acetate — glacial acetic acid — absolute ethanol 120 : 130 : 2 : 1 (v/v/v/v)
6 = silica gel-G, chloroform — dioxane 95 : 5 (v/v)
$1a_0$ = silica gel-G, cyclohexane — ethyl acetate 80 : 20 (v/v)
4 = silica gel-G, chloroform — acetone 3 : 2 (v/v)

was hydrolysed and then again chromatographed in system 4. PBA was likewise performed on the various portions of free Δ^5-diol purified in this way, and here too the final result was taken as the mean of the different measurements.

PBA was also used to measure the Test-S as free Test following solvolysis. The amounts of Test determined on various aliquots after its oxidation and reduction agreed with one another only within the limits of experimental error.

As regards the free steroids, identification of the 17-ketosteroids (A-anedione, Δ^4-dione, DHA and And) was described previously [7]. The free 17β-hydroxy-steroids (Δ^5-diol, Test and DHT) were identified as indicated in Table I.

The quantities of seven free androgen steroids in the axillary hair of sexually mature healthy males (6) and females (6) are listed in Table II. The Δ^5-diol, one of the most important precursors of Test, occurs in considerably higher amounts (females: 6.10 ± 3.10; males: 9.91 ± 3.13 nmol/g) than either Test (females: 0.32 ± 0.03; males: 0.91 ± 0.31 nmol/g) or DHT (females: 0.42 ± 0.07; males: 0.65 ± 0.17 nmol/g), each of which displayed high individual fluctuations in both sexes.

Of the 17-ketosteroids with weak androgen activity, DHA was found in the highest concentration in the axillary hair of both females and males (females: 19.83 ± 8.34; males: 31.82 ± 9.75 nmol/g). This was followed in order by Δ^4-dione (females: 1.85 ± 0.53; males: 17.85 ± 5.59 nmol/g) and A-anedione (females: 0.91 ± 0.31; males: 9.06 ± 5.04 nmol/g). The concentration of free And was surprisingly low (females: 0.60 ± 0.16; males: 1.52 ± 0.58 nmol/g), though the conjugated form of this steroid was found in appreciable quantities in axillary hair.

Table II

Amount of free androgenic steroids in axillary hair of healthy women and men (mean ± SEM)

Steroids	Women (21—40 years) n = 6	Men (21—41 years) n = 6
Δ^5-diol (nmol/g)	6.10 ± 3.10	9.91 ± 3.13
Test (nmol/g)	0.32 ± 0.03	0.91 ± 0.31
DHT (nmol/g)	0.42 ± 0.07	0.65 ± 0.17
DHA (nmol/g)	19.83 ± 8.34	31.82 ± 9.75
And (nmol/g)	0.60 ± 0.16	1.52 ± 0.58
Δ^4-dione (nmol/g)	1.85 ± 0.53	17.85 ± 5.59
A-ane-dione (nmol/g)	0.91 ± 0.31	9.06 ± 5.04

Table III

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

Subject No.	Sex	Age years	DHA-S μmol/g	And-S μmol/g	Δ^5-diol-S μmol/g	Test-S nmol/g
1	female	21	2.39	0.59	0.16	8.44
2	female	30	1.22	0.11	0.51	1.84
3	female	32	4.83	0.32	0.89	2.88
4	female	36	2.36	0.43	0.48	1.84
5	female	40	5.29	0.70	1.54	4.18
Limits			1.22 — 5.29	0.11 — 0.70	0.16 — 1.54	1.84 — 8.44
mean ± SEM			3.22 ± 0.78	0.43 ± 0.10	0.72 ± 0.24	3.83 ± 1.22
6	male	21	1.76	0.43	0.35	0.68
7	male	25	9.91	0.70	2.40	1.55
8	male	34	4.50	0.32	1.35	13.65
9	male	34	1.46	0.30	0.54	1.76
10	male	41	4.61	0.43	4.02	1.36
Limits			1.46 — 9.91	0.30 — 0.70	0.35 — 4.02	0.68 — 13.65
mean ± SEM			4.45 ± 1.52	0.43 ± 0.07	1.73 ± 0.68	3.80 ± 2.47

The difference between males and females was not significant statistically because of the high individual fluctuations; nevertheless, the mean values for the individual free steroids were considerably higher for the males.

Table III presents the quantities of four steroid sulphates in the axillary hair of sexually mature, healthy males (5) and females (5). Of the water-soluble C_{19}-steroid sulphates, DHA-S was found in the highest amount (females: 3.22 ± 0.78; males: 4.45 ± 1.52 μmol/g) followed by Δ^5-diol-S (females: 0.72 ± 0.24; males: 1.73 ± 0.68 μmol/g), And-S (females: 0.43 ± 0.10; males: 0.43 ± 0.07 μmol/g) and Test-S (females: 3.83 ± 1.22; males: 3.80 ± 2.47 nmol/g).

Discussion

The fact that the free steroids and the steroid sulphates can be extracted from axillary hair with a mixture of ammonium hydroxide and diethyl ether proves that the steroids are not incorporated into the substance of the hair.

Although the free steroids were found at low concentration in human axillary hair, their importance is not negligible, since each of them (particularly

the 17β-hydroxysteroids) is androgen active. Since the free steroids are soluble in fats, it is assumed that they originate from the sebum, a product of the sebaceous glands opening into the hair follicles.

In humans, stimulation of the sebaceous glands begins with puberty; sexually mature males produce more sebum than do females [12, 13]. In both humans and experimental animals the activity of the sebaceous glands is enhanced by Test, $\triangle^4$-dione and DHA, and inhibited by a high dose of oestrogen and cyproterone acetate [14, 15, 16].

The bulk of the steroids determined in axillary hair proved to be water-soluble steroid sulphates. DHA-S, And-S and Δ^5-diol-S are present in almost thousand times the amount of the free androgen steroids. Only the quantity of Test-S lies among the values for the free steroids. Since the lipoid layer coating human hair develops from products of the sebaceous and sweat glands, it may be assumed that the water-soluble steroid sulphates pass into the hair via the sweat.

With a gas chromatographic method, Karunakaran et al. [17] demonstrated numerous free and sulphate ester steroids in sebum collected from various surfaces of the body, and the dorsal and facial skin. They also found free C_{19}-steroids in nanogram quantities, in contrast with the data of Dubovi [18] and Oertel and Treiber [19], who reported that non-conjugated steroids are secreted by the sebaceous glands in microgram quantities.

The amounts of free and sulphate ester steroids determined in axillary hair varied within broad limits from individual to individual. This variability may be explained primarily by the different numbers and activities of the sebaceous and sweat glands in different individuals, but it may also be caused by individual differences in hygiene.

Our studies have shown the human skin excretes all those biologically active androgens (Test, DHT, And, etc.) which earlier were identified as metabolites in incubation experiments. It may be assumed that some of the androgen steroids detected in hair are products of the steroid metabolism occurring in the skin. The large quantity of C_{19}-steroid sulphates found in axillary hair have confirmed that the sweat glands have a considerably more important role in steroid excretion in the axillae than do the sebum-producing sebaceous glands.

REFERENCES

1. Faredin, I., Fazekas, Á. G., Tóth, I., Kókai, K., Julesz, M.: Transformation in vitro of [4-^{14}C] dehydroepiandrosterone into 7-oxygenated derivatives by normal human male and female skin tissue. J. Invest. Derm. **52**, 357–361 (1969).
2. Faredin, I., Kókai, K., Tóth, I., Fazekas, Á. G., Julesz, M.: The metabolism of [4-^{14}C]-dehydroepiandrosterone by human skin in vitro. I. Transformation in vitro of [4-^{14}C]-dehydroepiandrosterone into androst-4-ene-3,17-dione, testosterone and androsterone by normal human male and female skin slices. Acta Med. Acad. Sci. Hung. **27**, 95–102 (1970).

3. Faredin, I., Tóth, I., Julesz, M.: The metabolism of [4-^{14}C]dehydroepiandrosterone by human skin in vitro. II. In vitro formation of water-soluble dehydroepiandrosterone sulphate and androst-5-ene-3β,17β-diol-3-sulphate in human abdominal skin. Acta Med. Acad. Sci. Hung. **30,** 91–99 (1973).
4. Faredin, I., Tóth, I., Fazekas, Á. G., Kókai, K., Julesz, M.: The in vitro metabolism of [4-^{14}C]androst-4-ene-3,17-dione by normal and hirsute female skin. Int. J. Derm. **9,** 147–152 (1970).
5. Faredin, I., Tóth, I.: The metabolism of [4-^{14}C]5-androstene-3β,17β-diol by normal human skin in vitro. Acta Med. Acad. Sci. Hung. **32,** 139–152 (1975).
6. Julesz, M., Faredin, I., Tóth, I.: Steroids in human skin and hairs. IV. Neutral 17-ketosteroids in human hairs. Acta Med. Acad. Sci. Hung. **22,** 49–52 (1966).
7. Julesz, M., Faredin, I., Tóth, I.: Steroids in Human Skin. Akadémiai Kiadó, Budapest 1971.
8. Tóth, I., Faredin, I.: Separation and identification of free and esterified 17-ketosteroids. Acta Med. Acad. Sci. Hung. **29,** 141–144 (1972).
9. Treiber, L. R.: Zur Bestimmung von Ketosteroiden mit 2,4-dinitrophenylhydrazin. Inaugural-Dissertation. Universität des Saarlandes, Mainz 1968.
10. Tóth, I., Faredin, I.: A testosteron, az 5α-dihydrotestosteron, az 5-androsten-3β,17β-diol és a 4-androsten-3,17-dion szimultán meghatározása emberi serumból (Simultaneous assay of testosterone, 5α-dihydrotestosterone 5-androstene-3β,17β-diol and 4-androstene-3,17-dione in human serum). Kísérl. Orvostud. **30,** 55–65 (1978).
11. Tóth, I., Faredin, I.: Androgen steroids in human axillary hair lipids. Acta Physiol. Acad. Sci. Hung. **51,** 209–210 (1978).
12. Strauss, J. S., Pochi, P. E.: The human sebaceous gland: its regulation by steroidal hormones and its use as an end organ for assaying androgenicity in vivo. Recent Progr. Hormone Res. **19,** 385–444 (1963).
13. Nikkari, T.: Composition and secretion of the skin surface lipids of the rat; effects of dietary lipids and hormones Scand. J. Clin. Lab. Invest. **17,** Suppl., 85 (1965).
14. Pochi, P. E., Strauss, J. S.: Sebaceous gland response in man to the administration of testosterone, Δ^4-androstenedione and dehydroepiandrosterone. J. Invest. Derm. **52,** 32–36 (1969).
15. Ebling, F. J.: Hormonal control and methods of measuring sebaceous gland activity. J. Invest. Derm. **62,** 161–171 (1974).
16. Ebling, F. J., Thomas, A. K., Cooke, L. D., Randall, V. A., Skinner, J., Cawood, M.; Effect of cyproterone acetate on hair growth, sebaceous secretion and endocrine parameters in a hirsute subject. Brit. J. Derm. **97,** 371–381 (1977).
17. Karunakaran, M. E., Pochi, P. E., Strauss, J. S., Valerio, E. A., Wotiz, H. H., Clark, S. J.: Androgens in skin surface lipids. J. Invest. Derm. **60,** 121–125 (1973).
18. Dubovi, M. J.: Androgenic steroids in the sebum. Vestn. Derm. Vener. **41,** 14–21 (1967).
19. Oertel, G. W., Treiber, L.: Metabolism and excretion of C_{19}- and C_{18}-steroids by human skin. Eur. J. Biochem. **7,** 234–238 (1969).

Acta Medica Hungarica 40 (2—3), pp. 147—152 (1983)

Hepatology

HEPATITIS A VIRUS ANTIBODY IN CHRONIC DIFFUSE LIVER DISEASE

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(Received February 9, 1982)

175 patients with histological evidence of chronic diffuse liver disease, 67 patients with heart failure, diabetes and atherosclerosis, and 118 healthy adults under 30 years of age engaged in sports were studied for the prevalence of hepatitis A virus antibody (anti-HAV) by radioimmunoassay using a HAVAB (Abbott)-kit. Infection with hepatitis-A virus is highly prevalent in Hungary, anti-HAV having been demonstrated in a very high proportion of controls as well as of patients. Over the age of 40 the incidence is 100% in controls and 98% in patients with chronic liver disease. Infection with hepatitis-A virus must have been asymptomatic in the majority, since no more than 11.4% of the subjects had a history of acute hepatitis. The prevalence of acquired anti-HAV increases with age until it attains 100% in advanced age. The present results lend no support to the possibility that hepatitis-A virus infection might be involved in the production of chronic diffuse liver disease.

Keywords: hepatitis-A, hepatitis A antibody, chronic liver disease

Introduction

Virus A hepatitis is a well-known disease, and its route of transmission is also known [4]. Yet our knowledge about the responsible virus was very limited until research in this field had received new impetus by the immuno electron microscopic demonstration of the hepatitis-A-virus (HAV) in 1973 [6]. Since that time numerous methods have been developed for the study of HAV and anti-HAV [7, 9, 13, 14, 16, 19, 20] and it has been shown that the prevalence of HAV infection is very high all over the world, anti-HAV having been demonstrated in 17 to 97% of the populations studied [8, 10, 25, 27]. Data from Hungary have not been available, in the neighbouring countries Yugoslavia and Austria the figures for anti-HAV positivity were found to attain 97 and 70%, respectively [22, 27]. In the majority of the cases HAV infection causes no symptoms, since indications of an acute hepatitis in the history were confined to 3 to 10% of the anti-HAV-positive subjects [8, 26]. HAV infection is far more prevalent in poor social classes or in populations

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living under crowded conditions with inadequate sanitation than those of high standards [26, 28].

A chronic HAV carrier state has not been ascertained thus far [21, 26], but recent immunofluorescent studies of liver tissue and radioimmunoassay for IgM-anti-HAV indicate that HAV infection may persist far longer than it was believed [7, 14]. In the majority of the cases complete cure of acute HA ensues, but occasionally the laboratory findings are abnormal several months after infection, and production of chronic hepatitis after acute HA has also been described [17, 18, 24].

In view of these observations and of the high frequency of HAV infection we intended to examine the following questions;

1. Is there any relationship between advancing age and the incidence of HAV infection?

2. In what proportion of the various types of chronic diffuse liver disease has an earlier HAV infection occurred?

3. Is there any connection between chronic diffuse liver disease and HAV infection?

Patients and methods

One hundred and 75 patients with chronic diffuse liver disease were studied. The diagnosis was confirmed by biopsy, and the histological features were classified on the basis of the internationally accepted morphological criteria [1, 3, 5]. In 60 patients fatty degeneration of the liver (FD), in 29, chronic persistent hepatitis (CPH), in 27, chronic active hepatitis (CAH), in 35, liver cirrhosis (LC) were present and in 24 patients alcoholic liver disease, subacute hepatitis, non-specific reactive hepatitis had been diagnosed. Distribution of the patients by age and diagnosis is shown in Table I. The controls were 67 inpatients, aged from 30 to

Table I

Distribution of patients by age and diagnosis

Diagnosis	Under 20	20–29	30–39	40–49	50–59	Over 60	Total No.
Fatty degeneration	0	3	8	29	18	2	60
CPH	1	1	3	9	11	4	29
CAH	0	1	4	9	8	5	27
Cirrhosis	0	0	2	10	11	12	35
Other*	1	5	6	7	3	2	24
Total	2	10	23	64	51	25	175
Controls	118		11	11	19	33	192

* Alcoholic hepatitis, subacute hepatitis, non-specific reactive hepatitis

80 years, being under treatment for heart failure, diabetes, atherosclerosis, etc., and 118 healthy subjects under 30 years of age, engaged in sports. The age distribution of the controls is seen in Table I.

The anti-HAV was measured in the sera by HAVAB RIA kit made by Abbott Laboratories.

Results

Results in the controls are shown in Table II. It can be seen that under the age of 30 years the proportion of positive cases was low but it increased considerably with advancing age. Beyond the fourth decade of life 100% of the subjects studied were anti-HAV positive.

The frequency of anti-HAV positivity in chronic diffuse liver disease is presented in Table III. Anti-HAV positivity has been demonstrated in 160 out of 175, i.e. 91% of the patients. The proportion of anti-HAV positive sera was very high in all types of liver disease.

The occurrence of acute hepatitis is seen as Table IV shows. Acute hepatitis was mentioned in the history by 11.4% of the 175 cases, the proportion of patients with previous hepatitis was highest in CAH and LC. None of the anti-HAV negative patients had a history of acute hepatitis (Table IV).

Table II

Age-related prevalence of anti-HAV positivity in controls

	Under 30	30–39	40–49	50–59	Over 60
Total No.	118	11	11	19	33
Positive, No.	7	7	11	19	33
Per cent	6	64	100	100	100

Table III

Prevalence of anti-HAV in chronic diffuse liver disease

Diagnosis	Positive	
	No.	%
Fatty degeneration (n = 60)	56	93
CPH (n = 29)	24	83
CPH (n = 27)	25	93
Cirrhosis (n = 35)	35	100
Other (n = 24)	20	83

Table IV

Proportion of patients with a history of acute hepatitis

Diagnosis	Anti-HAV positive patients (n = 160)		Anti-HAV negative patients (n = 15)
	No.	%	
Fatty degeneration (n = 60)	4	6.7	0
CPH (n = 29)	3	10.3	0
CAH (n = 27)	5	18.5	0
Cirrhosis (n = 35)	6	17.1	0
Other (n = 24)	2	8.3	0
Total (n = 175)	20	11.4	0

Figure 1 shows the age-related distribution of anti-HAV positivity in liver disease. Under 30 years of age 33%, between 30 and 39 years, 78%; between 40 and 49 years, 97%; and beyond 50 years, 100% of the patients were found anti HAV positive.

Discussion

The differences in the figures of anti-HAV positivity, i.e. of earlier HAV infection, between the patients with chronic diffuse liver disease and the control group (except group under 30 years of age), as also between the individual groups of liver disease, were negligibile. This is consistent with the results of Lindberg et al. [11] and Renner et al. [22] obtained in patients with CAH and in blood donors. Our observation that a history of earlier hepatitis was confined to 11.4% of the patients indicates that a HAV infection is asymptomatic in the majority of cases. The method used in this study for the demonstration of anti-HAV is unsuited for differentiation between IgG and IgM antibodies. This is, however, important, since the presence of IgM-anti-HAV in the serum reflects a persisting infection, whereas IgG-anti-HAV points to a past infection [2, 12]. The sensitive IgM-anti-HAV radioimmunoassay allowed to identify this antibody in a number of cases as late as 6 to 12 months after infection [7, 23]. Despite this fact it has not been possible to prove the existence of an actual HAV carrier state, in other words, to furnish evidence connecting the production of chronic diffuse liver disease with an earlier HAV infection [15, 21, 29].

In the present material the proportion of anti-HAV positive cases, in other words, the occurrence of earlier infection, has been found to increase with advancing age. Similar figures have been reported for European and North-

American populations, where the living conditions are similar to those in Hungary [8, 25, 26, 30]. In populations living under crowded conditions with poor sanitation the figures for anti-HAV positivity are very high even in young age groups and show no further increase over later years [8, 26, 27, 28].

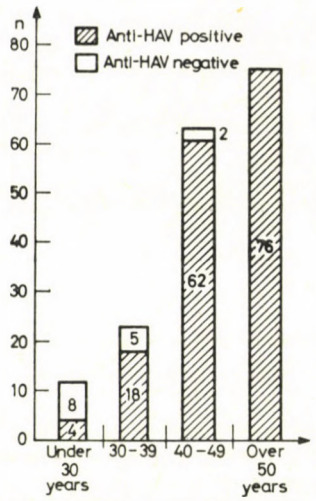

Fig. 1. Age related prevalence of anti-HAV-positivity in chronic diffuse liver disease

From the present observations the following conclusions may be drawn.

1. The proportion of subjects with evidence of earlier HAV infection increases parallel with age until it attains 100%.

2. The incidence of HAV infection is high in Hungary, and the course of the infection is asymptomatic in most cases. The absence of any difference in the figures of anti-HAV positivity between the patients with liver disease and the controls indicates that HAV infection plays no part in the production of chronic diffuse liver disease.

Acknowledgements

We are indebted to Mrs. I. Erdélyi and Mrs. K. G. László for radioimmunoassays and to Dr. J. T. Kelemen and to Dr. Gy. Stotz for histological studies.

REFERENCES

1. Anthony, P. P., Ishak, K. G., Nayak, N. L.: The morphology of cirrhosis: definition, nomenclature and classification. Bull. WHO **55**, 521–540 (1977).
2. Bradley, D. W., Maynard, J. E., Hindman, S. H., Hornbeck, C. L., Fields, H. A., McCaustland, K. A., Cook, E. H.: Serodiagnosis of acute phase immunoglobulin M anti-hepatitis A virus by radioimmunoassay. J. Clin. Microbiol. **5**, 521–530 (1977).

3. Bianchi, L., De Groote, J., Desmet, V. J., Gedick, P., Korb, G., Popper, H., Poulsen, H., Scheuer, P. J., Schmid, M., Thaler, H., Wepler, W.: Acute and chronic hepatitis revisited. Lancet **2,** 914–919 (1977).
4. Cossart, Y. E.: The virus hepatitis A. In: Virus Hepatitis and its Control. Bailiere Tindall, London 1977, pp. 8–19.
5. De Groote, J., Desmet, V. J., Gedick, P., Korb, G., Popper, H., Poulsen, H., Scheuer, P. J., Schmid, M., Thaler, H., Uehlinger, E., Wepler, W.: A classification of chronic hepatitis. Lancet **2,** 626–628 (1968).
6. Feinstone, S. M., Kapikian, A. Z., Purcell, R. H.: Hepatitis A: detection by immune electron microscopy of a virus-like antigen associated with acute illness. Science **182,** 1026–1028 (1973).
7. Flehmig, B., Ranke, M., Berthold, H., Gerth, H. J.: A solid-phase radioimmunoassay for detection of IgM antibodies to hepatitis A virus. J. Infect. Dis. **140,** 169–175 (1979).
8. Froesner, G. G., Froesner, H. R., Dietz, H. H., Dietz, K., Sugg, H., Schneider, W.: Häufigkeit von Hepatitis A Antikörpern in Bevölkerungsgruppen verschiedener Europäischer Länder. Schweiz. Med. Wschr. **107,** 129–133 (1977).
9. Hollós, I., Pálfi, A., Baranyai, E., Novák, E.: Immune-Indian ink method for the detection of hepatitis A associated antigen and antibody. Acta Microbiol. Acad. Sci. Hung. **22,** 169–178 (1975).
10. Lehman, N. I., Gust, I. D.: The prevalence of antibody to hepatitis A virus in two populations in Victoria. Med. J. Aust. **2,** 731–732 (1977).
11. Lindberg, J., Froesner, G., Hansson, B. G.: Serologic markers of hepatitis A and B in chronic active hepatitis. Scand. J. Gastroent. **13,** 525–527 (1978).
12. Locarnini, S. A., Ferris, A. A., Lehman, N. J., Gust, I. D.: The antibody response following hepatitis A infection. Intervirology **8,** 309–318 (1977).
13. Mathiesen, L. R., Feinstone, S. M., Wong, D. C., Skinhoj, P., Purcell, R. H.: Enzyme linked immunosorbent assay for detection of hepatitis A antigen in stool and antibody to hepatitis A antigen: comparison with solid phase radioimmunoassay, immune electron microscopy and immune adherence haemagglutination assay. J. Clin. Microbiol. **7,** 184–193 (1978).
14. Mathiesen, L. R., Fauerholdt, L., Moller, A. M., Aldershvile, J., Dietrichson, O., Hardt, F., Nielsen, J. O., Skinhoj, P.: Immunfluorescence studies for hepatitis A virus and hepatitis B surface and core antigen in liver biopsies from patients with acute viral hepatitis. Gastroenterology **77,** 623–628 (1979).
15. Mathiesen, L. R., Hardt, F., Dietrichson, O.: The role of acute hepatitis type A, B and non-A, non-B in the development of chronic active liver disease. Scand. J. Gastroent. **15,** 49–54 (1980).
16. Müller, W. J., Provost, P. J., McAlleen, W., J., Iffensohn, O. L., Villarejos, V. M.: Specific immune adherence assay for human hepatitis A antibody. Proc. Soc. Exp. Biol. (N. Y.) **149,** 254–261 (1975).
17. Mosley, J. W., Galambos, J. T.: Viral hepatitis. In: Schiff, L. (ed.): Diseases of the Liver. Lippincott, Philadelphia 1975, pp. 500–593.
18. Norkrans, G., Froesner, G., Hermodsson, S., Iwarson, S.: Clinical, epidemiological and prognostic aspects of hepatitis "non A, non B" in comparison with hepatitis A and B. Scand. J. infect. Dis. **11,** 259–264 (1979).
19. Provost, P. J., Iffensohn, O. L., Villarejos, V. M., Hilleman, M. R.: A specific complement fixation test for human hepatitis A employing CR 326 virus antigen: diagnosis and epidemiology. Proc. Soc. Exp. Biol. (N. Y.) **148,** 962–969 (1975).
20. Purcell, R. H., Wong, D. C., Moritsugu, Y., Dienstag, J. L., Routenberg, J. A., Boggs, J. D.: A microtiter solid phase radioimmunoassay for hepatitis A antigen and antibody. J. Immunol. **116,** 349–356 (1976).
21. Rakela, J., Redeker, A. G., Edwards, W. M., Becker, R., Overby, L. R., Mosley, J. W.: Hepatitis A virus infection in fulminant hepatitis and chronic active hepatitis. Gastroenterology **74,** 879–882 (1978).
22. Renner, F., Horak, W., Grabner, G., Dittrich, H.: Zur Ätiologie der chronisch aggressiven Hepatitis in Wien. Z. Gastroent. **17,** 106–109 (1979).
23. Roggendorf, M., Froesner, G. G., Deinhardt, F., Scheid, R.: Comparison of solid phase test system for demonstrating antibodies against hepatitis A virus (anti-HAV) of the IgM class. J. Med. Virol. **5,** 47—62 (1980).
24. Routenberg, J. A., Dienstag, J. L., Harrison, W. O., Kilpatrick, M. D., Hooper, R. R., Purcell, R. H.: A foodborne epidemic of hepatitis A virus (HAV) infection among navy recruits. Gastroenterology **69,** 859 (1975).

25. Stadelman, W., Gasser, J., Löffler, H.: Die Häufigkeit von Antikörpern gegen Hepatitis-A-Virus bei verschiedenen Altersgruppen der Basler Bevölkerung 1978/79. Schweiz. Med. Wschr. **110,** 975–978 (1980).
26. Szmuness, W., Dienstag, J. L., Purcell, R. H., Harley, B. J., Stevens, C. E., Wong, D. C.: Distribution of antibody to hepatitis A antigen in urban adult populations. New Engl. J. Med. **295,** 755–759 (1976).
27. Szmuness, W., Dienstag, J. L., Purcell, R. H., Stevens, C. E., Wong, D. C., Ikram, H., Bar-shany, S., Beasley, R. P., Desmyter, J., Gaon, J. A.: The prevalence of antibody to hepatitis A antigen in various parts of the world: a pilot study. Amer. J. Epidemiol. **106,** 392–398 (1977).
28. Szmuness, W., Purcell, R. H., Dienstag, J. L., Stevens, C. E.: Antibody to hepatitis A antigen in institutionalized mentally retarded patients. J. Amer. Med. Ass. **237,** 1702–1705 (1977).
29. Szmuness, W., Dienstag, J. L., Purcell, R. H., Prince, A. M., Stevens, C. E., Levine, R. W. Hepatitis type A and hemodialysis. A seroepidemiological study in 15 U.S. centers. Ann. Intern. Med. **87,** 8–12 (1977).
30. Vogten, A. J. M., Siegenbeck van Henkelom, L. H., Kater, L.: Frequency and clinical significance of hepatitis A antibodies in the Netherlands. Neth. J. Med. **22,** 10–12 (1979).

Acta Medica Hungarica 40 (2—3), pp. 155—162 (1983)

SERUM ALPHA-FETOPROTEIN IN CHRONIC LIVER DISEASE

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(Received August 4, 1982)

The serum alpha-fetoprotein level was measured by radioimmunoassay in 20 normal subjects and in 118 patients with chronic liver disease. The highest concentrations were found in the group of hepatocellular carcinoma. Compared with the controls, higher values were demonstrable in the other groups of liver disease as well. This is attributed to the more or less marked regeneration of liver tissue, which is never absent in any type of liver disease studied, and in the case of fatty degeneration, to the alpha-fetoprotein inducing effect of alcohol. The importance of alpha-fetoprotein estimation in the diagnosis of hepatocellular carcinoma and monitoring of its therapy, as also in the assessment of activity and regeneration in other forms of chronic liver disease, are pointed out. Countercurrent electrophoresis has been found unreliable for the measurement of alpha-fetoprotein.

Keywords: serum alpha-fetoprotein, chronic liver diseases, regeneration of liver tissue

Abbreviations

AFP = serum alpha-fetoprotein, AP = alkaline phosphatase, CAH = chronic active hepatitis, CLD = chronic liver disease, CL = cirrhosis of liver, CPH = chronic persistent hepatitis, ELISA = enzyme-immunoassay, GGT = gamma-glutamyl-transferase, HBsAg = hepatitis B viral surface antigen, HCC = hepatocellular carcinoma, LD = liver disease, LM = extrahepatic tumour with liver metastases, SGOT = = serum-aspartate-aminotransferase, FD = alcoholic, non-inflammatory fatty degeneration of liver, RIA = radioimmunoassay

Introduction

Embryonal antigens are tumour antigens present during fetal development, but occurring at low concentrations also in normal adult sera. Certain diseases may be associated with significantly increased serum levels, therefore their demonstration may have a diagnostic value [15].

Alpha-fetoprotein (AFP) is a fetal antigen of alpha-globulin nature, the postnatal production of which is practically confined to the hepatocytes [16, 17, 18]. The serum level is high during intrauterine life, but falls rapidly after birth. In healthy adults the serum AFP level is under 10 μg/l, with the exception of the second and third trimesters of pregnancy, when its concentration in the maternal blood increases, owing to fetal production [16, 19, 26].

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Production of AFP by cells of certain embryonal tumours such as hepatocellular carcinoma (HCC) or ovarian and testicular teratoma was first reported by Abelev [1] and Tatarinov [46], and confirmed later by other authors [7, 8, 28, 29, 32, 38, 51]. Occasionally cells of other tumours, first of all those of the digestive tract, pancreas and bronchi, display an AFP producing capacity [28, 47, 51].

Measurement of the AFP level in maternal serum is indispensable today for the early detection of certain anomalies, in the first place of neural tube defects [5, 11, 14, 36, 48].

Production of AFP is, however, not confined to fetal and malignant cells, as confirmed by immunofluorescent assays, the oval and intermediate cells formed in the course of liver tissue regeneration also have this capacity [31, 33, 41, 49]. High serum AFP levels in chronic non-malignant liver disease have been reported by numerous authors [7, 12, 13].

Experimental proof that certain agents may stimulate mature liver cells to AFP production as a result of activation of the AFP-specific gene, has been furnished by Taketa et al. [45] and Watanabe et al. [52].

Measurement of AFP as a tumour marker or as a diagnostic aid in non-malignant liver disease associated with AFP production is therefore of practical value. There are various methods for this purpose: immuno electrophoresis, radioimmunoassay (RIA), enzyme-immunoassay (ELISA) or micro-ELISA. AFP can be visualized in living tissues by means of I^{131}-labelled anti-AFP antibodies of the IgG type by means of a gamma camera [20, 21, 34, 35, 36].

In the present study we have measured AFP in the sera of patients with chronic liver disease (CLD) in order to find out whether countercurrent electrophoresis was suitable for the replacement of RIA in screening of HCC, generally associated with a high serum AFP level and whether this assay lends itself to the assessment of regeneration and of the activity of liver disease (LD).

Patients and methods

In normal controls (10 females and 10 males, aged 24–42 years) and 118 patients with LD (54 females females and 64 males, aged 27–73 years) the serum AFP concentration was estimated by RIA, using an Amersham kit. The sera of 4 controls and 40 patients were studied by countercurrent electrophoresis as well, using the electrophoresis equipment and Radiophor kit of IMMUNO.

All patients had chronic, diffuse LD. Distribution according to diagnosis was as follows. Alcoholic, non-inflammatory fatty degeneration (FD) in 10, chronic persistent hepatitis (CPH) in 6, chronic active hepatitis (CAH), treated and in an inactive period all throughout, in 12, cirrhosis of liver (CL) in 55, hepatocellular carcinoma (HCC) in 6 and extrahepatic tumour with liver metastases (LM) in 29 cases. In the last group there had been clinical signs of liver deposits, and evidence of diffuse malignant infiltration of the liver was furnished by necropsy.

The clinical diagnosis was confirmed by the laboratory findings, biopsy, and in some of the cases by necropsy.

Assessment of activity was based on the clinical picture and on the laboratory findings, first of all on the serum-aspartate-aminotransferase (SGOT) value. The process was not con-

sidered active unless SGOT attained or exceeded 60 U/l. The serum AFP concentration was regarded as increased from 10 μg/l upward.

Ten of the patients with CL were checked for serum AFP in an active, as well as an inactive period of the disease.

All patients were studied for SGOT (normal maximum 20 U/l), gamma-glutamyl-transferase (GGT, normal maximum 25 U/l), alkaline phosphatase (AP, normal maximum 80 U/l) and for hepatitis B viral surface antigen (HB_sAg), using an Ausria-II kit Abbott.

For statistical analysis Student's two sample *t* test and linear regression analysis were used.

Results

The mean serum AFP concentration in the control group was 2.21 ± 0.27 (SE) μg/l. No noteworthy differences in AFP were found between male and female values.

The serum AFP values of the subjects are represented in Fig. 1.

The highest AFP values were found in the HCC group, but the mean AFP concentration in the other groups was also higher than in the control group.

CL patients in the active period of the disease had significantly higher serum AFP levels than did those in the inactive period ($P < 0.001$) and the same patient in the periods of activity and inactivity gave the same relation-

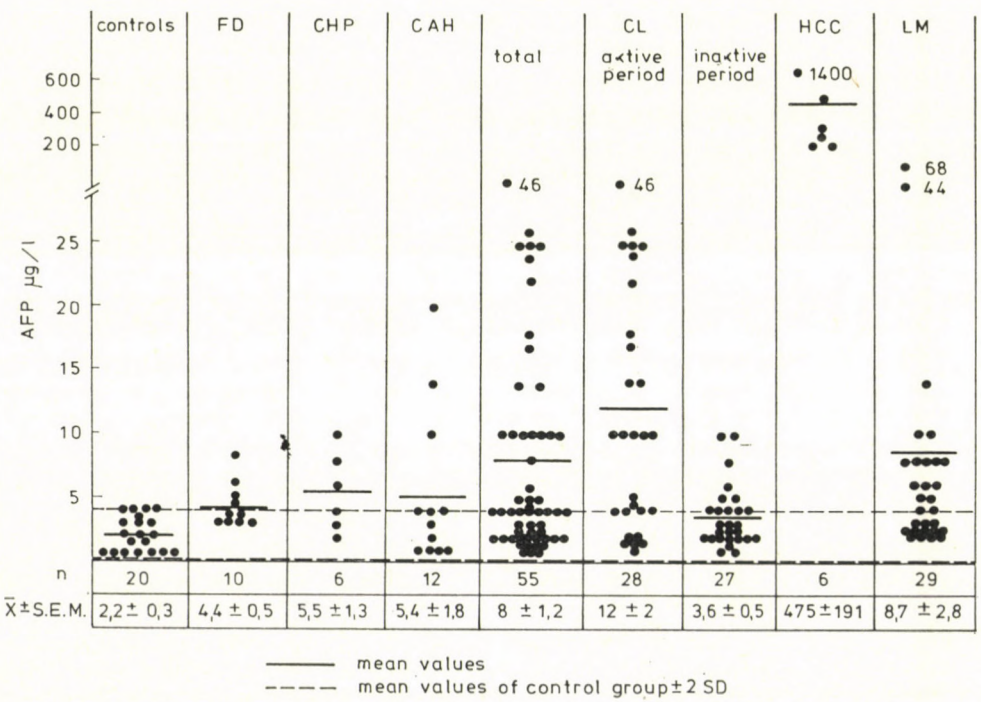

Fig. 1. Serum AFP values of patients

ships. In 8 of the 10 patients the values found during the active period of CL were greatly in excess of those measured during the inactive period. The changes in activity of the process in either direction were closely followed by

Table I

AFP values in 10 patients with liver cirrhosis, in active and inactive periods

No.	Sex	AFP μg/l	
		active period	inactive period
1.	M	14	4
2.	M	4	4
3.	F	17	2
4.	M	18	1.5
8.	M	4.5	2
6.	M	24	5
7.	M	10	3
8.	F	2	2
9.	F	26	4
10.	F	14	3
Mean ± S.E.M.		13.4 ± 2.6	3.1 ± 0.4

$P < 0.005$

Table II

Correlation of SGOT to AFP in liver diseases associated with regeneration (CPH, CAH, CL, LM)

SGOT level	No.	AFP mean ± S.E.M. μg/l
≥60 U/l	38	10.5 ± 1.6
<60 U/l	64	6.1 ± 1.2

$P < 0.05$

Table III

Correlation of AFP to SGOT in liver diseases associated with regeneration (CPH, CAH CL, LM)

AFP level	No.	SGOT mean ± S.E.M. U/l
>10 μg/l	16	74 ± 8.1
≤10 μg/l	86	48.9 ± 5.4

$P < 0.02$

Table IV

Distribution of (and correlation between) AFP and HBsAg (n = number of patients)

	Controls (n = 20)	FD (n = 10)	CPH (n = 6)	CAH (n = 12)	CL (n = 55)	HCC (n = 6	LM (n = 29)	Total No.
AFP > 10 μg/l								
HB_sAg +	0	0	0	1	2	1	1	5 (23%)
HB_sAg —	0	0	0	1	9	5	2	
AFP ≷ 10 μg/l								
HB_sAg +	1	0	0	2	6	0	0	8 (8.3%)
HB_sAg —	19	10	6	8	38	0	26	88
Total HB_sAg +	1 (5%)	0	0	3 (25%)	8 (15%)	1 (17%)	1 (3.4%)	13 (11%)
HB_sAg —	19	10	6	9	47	5	28	105

those of the serum AFP level but in two cases there was no change whatever (Table I).

In the 102 patients with CLD marked by liver regeneration (CPH, CAH, CL, LM) with a SGOT activity ≷ 60 U/l the serum AFP concentrations were significantly higher than in the case of a moderate or normal SGOT activity (Table II). On the other hand, in patients with a high serum AFP level (> 10 μg/l) the SGOT was significantly higher than in the patients with a normal AFP level (Table III).

On examining the AFP against the SGOT values in each of the 102 cases referred to above it was found that in 82% (n = 60) of those with higher (double or more) AFP values than those of the controls, the SGOT activity was also higher than normal, furthermore, that in 66% (n = 75) of those with SGOT values at least twice as high as the control figures the serum AFP concentration was also higher than in the control group. The calculations revealed a positive but somewhat loose correlation between the serum AFP and SGOT levels in the CL group: the correlation coefficient r was 0.314. In the other disease groups, too, the correlation of AFP to SGOT was positive but still less complete, maximum r being 0.225. No noteworthy correlations were found between AFP and AP and GGT in any of the groups, r being in the neighbourhood of zero.

In non-biliary (secondary) cirrhosis (n = 47) the mean serum AFP concentration was 8.06 ± 1.37 μg/l, while in the biliary type it was 5.01 ± 1.15 μg/l. The difference was not significant.

In the group with a high (> 10 μg/l) serum AFP concentration the number of HB_sAg positive patients was significantly higher than in the group with normal serum AFP. In CPH, CL and HCC, too the number of HB_sAg positive

patients was higher among those with a high serum AFP level. Distribution of the Hb_sAg positive cases and the correlation between AFP and Hb_sAg are presented in Table IV.

Additional countercurrent electrophoresis performed in 40 cases failed to detect AFP, with the only exception of one patient with HCC, who had the highest AFP value, one of 1400 μg/l.

Discussion

The value of AFP measurement for the early diagnosis of HCC and monitoring of its therapy is no longer in doubt [1, 2, 7, 8, 24, 29, 32, 38, 46, 51]. In this study too, the highest AFP concentrations were found in HCC. All patients with HCC (only 6 in number) had very high AFP values, though, according to the literature, in 5 to 25% of the HCC patients the serum AFP concentration is within the normal range [3, 4, 24, 27, 30, 31, 51]. In fact, the AFP-producing capacity of the tumour cells is related to their specific differentiation [3], and this differentiation is of variable degree. This has been also confirmed by Balogh et al. [7] on the evidence of immunoperoxidase studies of liver tissue.

Though the involvement of HB_sAg in the aetiology of HCC, and the importance of a regular follow-up of HB_sAg positive patients with chronic LD for serum AFP have been duly emphasized, the published figures of HB_sAg positivity in HCC show very wide variations, i.e. between 15% and 75% [40, 50]. The present results agree best with those of Balogh et al. [7], who found HB_sAg positivity in 25% of their patients with HCC and in 8.9% of those with CL. The number of the present cases was obviously too small to be conclusive.

The value of AFP studies in non-malignant CLD (unrelated to HCC) is less clearly established. The importance of AFP measurements in liver diseases marked by the appearance of young cells capable of AFP production (CPH, CAH, CL, LM) for the assessment of regeneration, is well documented in the literature [1, 6, 7, 9, 10, 12, 13, 23, 37, 41, 42, 43, 53]. In the material of the present study formed by the last-named groups, patients with serum AFP concentrations greatly exceeding those of the controls are also represented.

Damage to the liver cells is reflected in an increased SGOT activity. A possible regeneration following upon hepatocellular destruction may bring AFP producing cells into existence. Increase in SGOT activity in an acute or fulminant liver process is, therefore, regarded as a sign of poor prognosis reflecting the failure of regeneration, unless it is accompanied or followed by an increase in the serum AFP concentration [6, 22, 44]. In chronic cases (CPH, CAH, CL, LM) serum AFP values tending to increase or persisting at

high levels are indicative of activity and regeneration. Excessive values reflect a malignant transformation [7, 9, 12, 13, 23, 43, 53]. Measurements of AFP, which have to be serial if necessary, may thus provide indirect information on the activity of liver disease, and indirect information on the tendency and extent of regeneration accompanying the process. According to Balogh et al. [7], Dávid and Halmy [12, 13] and Ruoslahti et al. [37], serum AFP is an indicator of the changes in the activity of LD. In the present study, too, significant differences have been found between the serum AFP concentrations in the active and inactive periods of CL. With increasing activity of the process the serum AFP concentration increases and as its activity subsides, the AFP returns to the initial value.

The correlations between serum AFP and other laboratory parameters have been extensively studied. While some authors found no close correlations [25], some others did [10, 12, 42]. According to Dávid and Halmy [13], there is a significant positive correlation between AFP and SGOT in CAH, and in CL AFP has been found to correlate significantly with AP and with GGT. In the CL group of the present material we found a positive, though somewhat loose correlation between AFP and SGOT. In the other patient groups the correlation was still less complete, although the SGOT values of those with high serum AFP concentrations and the serum AFP concentrations of those with high SGOT values were considerably higher than in the patients in whom these values were in the normal range. We found no correlation between AFP and the two other enzymes, AP and GGT. Observations regarding the correlation between AFP and the various laboratory parameters are thus inconsistent. One of the causes of this may lie in a failure of coincidence of the peak enzyme activity and the AFP level in the course of the destructive regenerative process. Moreover, the individual variability of the extent of regeneration, possibly its complete absence, has also to be taken into account.

In four of our patients with liver FD the serum AFP concentration exceeded that of the controls. Since in this process there is no regeneration, the high values may be attributed to an AFP-inducing activity of alcohol, although the opinions on this question are divided [10, 12, 45, 52].

A remarkable, though not significant, difference was found between serum AFP in non-biliary (secondary) and biliary (secondary) CL. The higher AFP values in the former group presumably reflect the more marked regenerative tendency of the non-biliary type of CL.

RIA is a reliable, accurate, sensitive, not too laborious method of assay and is therefore well suited for the measurement of the serum AFP concentration. It has, however, certain shortcomings: it is costly, requires special equipment and conditions. Countercurrent electrophoresis, though simple, is unsuited even for screening purposes in HCC suspect cases. In the present study

its positivity was confined to a single case of HCC with an extremely high AFP level. Pácsa and Pejtsik [34, 35, 36] have used the AFP-ELISA or micro-ELISA method to their satisfaction. The procedure is claimed to have the advantages of RIA without its costs and elaborate conditions.

In CLD the serum AFP level thus allows to assess the activity and the regenerative tendency of the process. Excessive values are suggestive of HCC.

REFERENCES

1. Abelev, G. I., Perova, S. D., Khramkova, N. I., Postnikova, Z. A., Irlin, I. S.: Production of embryonal alfa-globulin by transplantable mouse hepatomas. Transplantation **1,** 174–180 (1963).
2. Abelev, G. I., Assecritova, N., Kraevsky, N. A.: Embryonal $alfa_1$-globulin in cancer patients: diagnostic value. Int. J. Cancer **2,** 551–558 (1967).
3. Abelev, G. I.: Alfa-fetoprotein as a marker of embryospecific differentiation in normal and tumor tissues. Transplant. Rev. **20,** 3–37 (1974).
4. Alpert, M. E., Uriel, J., de Nechaud, B.: $Alpha_1$ and fetoglobulin in the diagnosis of human hepatoma. New Engl. J. Med. **278,** 948–986 (1968).
5. Balogh, T., Pejtsik, B., Ambrus, M., Bajtai, G., Páli, K.: Az $alpha_1$-foetoprotein meghatározásának jelentősége az intrauterin diagnosztikában. Orv. Hetil. **116,** 2887–2890 (1975).
6. Balogh, T., Bajtai, G., Ambrus, M., Péley, I., Kádas, I., Varga, L.: Az $alpha_1$-foetoprotein jelentősége a csecsemő és gyermekkori májsejtregenerátiós készség megítélésében. Orv. Hetil. **118,** 2815–2818 (1977).
7. Balogh, T., Bajtai, G., Ambrus, M., Pár, A., Palka, I.: Az $alpha_1$-foetoprotein szerepe a primaer hepatocelluláris carcinoma és egyes teratogen tumorok kórismézésében. Orv. Hetil. **121,** 2371–2374 (1980).
8. Bambert, P. O., Grob, P. J.: $Alpha_1$-fetoprotein and germ cell tumours. Schweiz. Med. Wschr. **109,** 1956–1962 (1979).
9. Bloomer, J. R., Waldman, T. A., McIntire, K. R., Klatskin, G.: Relationship of serum alpha-fetoprotein to the severity and duration of illness in patients with viral hepatitis. Gastroenterology **68,** 342–350 (1975).
10. Bloomer, J. R., Waldman, T. A., McIntire, K. R., Klatskin, G.: Alfa-fetoprotein in non-neoplastic liver disease. J. Amer. Med. Ass. **233,** 34–41 (1975).
11. Czeizel, E., Merétey, K., Osztovics, M., Sobel, M., Szőnyi, Gy.: A magzatvíz alfa-fetoprotein vizsgálatának jelentősége az anencephaliák-spina bifidák megelőzésében. Orv. Hetil. **116,** 1191–1191 (1975).
12. Dávid, K., Halmy, L.: A szérum $alfa_1$-foetoprotein koncentráció vizsgálata idült diffuz májbetegségekben. Orv. Hetil. **120,** 1677–1680 (1979).
13. Dávid, K., Halmy, L., Baranyai, P., Nagy, I.: Serum $alpha_1$-fetoprotein levels in chronic diffuse liver disease. Acta Med. Acad. Sci. Hung. **38,** 29–37 (1981).
14. Ferguson-Smith, M. A., May, H. M., Vince, J. D., Robinson, H. P., Rawlinson, H. A., Tait, H. A., Gibson, A. A. M., Ratcliffe, J. G.: Avoidance of anencephalic and spina bifida births by maternal serum alpha-fetoprotein screening. Lancet **1,** 1330–1333 (1978).
15. Gergely, J.: Tumorantigenek. Medicina, Budapest 1979. pp. 292–293.
16. Gitlin, D., Roesman, M.: Sites of serum alpha-fetoprotein synthesis in the human and in the rat. J. Clin. Invest. **46,** 1010–1016 (1967).
17. Gitlin, D., Perricelli, A.: Synthesis of serum albumin, prealbumin, alpha-fetoprotein, alpha-1-antitrypsin and transferrin by the human yolk, sac. Nature (Lond.) **228,** 995–997 (1970).
18. Gitlin, D.: Sites of alpha-fetoprotein synthesis. New Engl. J. Med. **285,** 1436–1437 (1971).
19. Gitlin, D.: Normal biology of alpha-fetoprotein. Ann. N. Y. Acad. Sci. **259,** 7–16 (1975).
20. Goldenberg, D. M., Kim, E. D., Deland, F., Spremulli, E., Nelson, M. O., Gockerman, J. P., Primus, F. J., Corgan, R. L., Alpert, E.: Clinical studies on the radioimmunodetection of tumours containing alpha-fetoprotein. Cancer **45,** 2500–2505 (1980).
21. Halmosdi, G., Bősze, P., László, J., Kovács, L.: A magzatvíz $alfa_1$-fetoprotein meghatározása immunoelectro-osmophoresissel hazailag előállított immunsavó felhasználásával. Orv. Hetil. **121,** 3049–3050 (1980).
22. Karvountzis, G. G., Redecker, A. G.: Relation of alpha-fetoprotein in acute hepatitis to severity and prognosis. Ann. Intern. Med. **80,** 156–160 (1974).

23. Kew, M. C., Purves, L. R., Bersohn, I.: Serum alphafetoprotein levels in acute viral hepatitis. Gut **14,** 939–942 (1973).
24. Kohn, J., Weaver, P. C.: Serum alpha$_1$-fetoprotein in hepatocellular carcinoma. Lancet **2,** 334–336 (1974).
25. Lehman, F. G.: Early detection of hepatoma: prospective study in liver cirrhosis using passive hemagglutination and radioimmunoassay. Ann. N. Y. Acad. Sci. **259,** 196–210 (1975).
26. Masseyeff, R., Bonet, C., Dronet, J., Sudaka, P., Lalanne, C.: Radioimmunoassay of alpha-fetoprotein I. Technique and serum levels in normal adults. Digestion **10,** 17–28 (1974).
27. McIntire, K. R., Vogel, C. L., Princler, G. L., Patel, I. R.: Serum alpha-fetoprotein as a biochemical marker for hepatocellular carcinoma. Cancer Res. **32,** 1941–1946 (1972).
28. McIntire, K. R., Waldman, T. A., Moertel, C. G.: Serum alpha-fetoprotein in patients with neoplasms of the gastrointestinal tract. Cancer Res. **35,** 991–996 (1975).
29. McIntire, K. R., Vogel, C. L., Primack, A., Waldman, T. A., Kyalwazi, S. K.: Effect of surgical and chemotherapeutic treatment on alpha-fetoprotein levels in patients with hepatocellular carcinoma. Cancer **37,** 677–683 (1976).
30. Melia, W. M., Johnson, P. J., Carter, S., Munro-Neville, A., Williams, R.: Plasma carcinoembryonic antigen in the diagnosis and management of patients with hepatocellular carcinoma. Cancer **48,** 1004–1008 (1981).
31. O'Connor, G. T., Tatarinov, Y. S., Abelev, G. I., Uriel, J.: Collaborative study for the evaluation of a serologic test for primary liver cancer. Cancer **25,** 1091–1098 (1970).
32. Okuda, K., Kotoda, K., Obata, H., Hayaschi, N., Hisamitsu, T., Tamiya, M., Kubo, Y., Yakushiji, F., Nagata, E., Jinnouchi, S., Shimokawa, Y.: Clinical observations during relatively early stage of hepatocellular carcinoma, with special reference to serum alpha-fetoprotein levels. Gastroenterology **69,** 226–234 (1975).
33. Onoe, N., Kanako, A., Dempo, K., Ogawa, K., Minase, T.: Alpha-fetoprotein and early histological changes of hepatic tissue in DAB-hepatocarcinogenesis. Ann. N. Y. Acad.
34. Pácsa, S., Pejtsik, B.: Enzim kapcsolatú technika vírus-specifikus ellenanyagok kimutatására. Orv. Hetil. **119,** 2071–2073 (1978).
35. Pácsa, S., Pejtsik, B.: Szérum alfa-foetoprotein szint meghatározása enzim kapcsolatú ellenanyag (Elisa) technikával normális és pathologiás terhességekben. Orv. Hetil. **121,** 151–154 (1980).
36. Pejtsik, B., Pácsa, S.: Velőcső-záródási rendellenességek szűrővizsgálata anyai szérum alfa-foetoprotein szint alapján, enzimkapcsolatú ellenanyag (ELISA) technikával. Orv. Hetil. **121,** 505–506 (1980).
37. Ruoslahti, E., Salaspuro, M., Pihko, H., Andersson, L., Seppala, M.: Serum alpha-fetoprotein: Diagnostic significance in liver disease. Brit. Med. J. **2,** 527–529 (1974).
38. Ruoslahti, E., Seppala, M.: Alpha-fetoprotein in normal human serum. Nature (Lond.) **235,** 161–162 (1972).
39. Sakamoto, S., Yachi, A., Anzai, T., Wada, T.: AFP-producing cells in hepatitis and liver cirrhosis. Ann. N. Y. Acad. Sci. **259,** 253–258 (1975).
40. Sakurai, M., Miyaji, T.: Alpha-fetoprotein and hepatitis B antigen in hepatocarcinogenesis. Ann. N. Y. Acad. Sci. **259,** 156–167 (1975).
41. Sell, S., Becker, F. F., Leffert, H. L., Watabe, H.: Expression of an oncodevelopmental gene product (alpha-fetoprotein) during fetal development and adult oncogenesis. Cancer Res. **36,** 4239–4249 (1976).
42. Silver, H. K. B., Gold, P., Shuster, J., Javit, N. B., Freedman, S. O., Finlayson, N. D. C.: Alpha$_1$-fetoprotein in chronic liver disease. New Engl. J. Med. **291,** 506–508 (1974).
43. Silver, H. K. B., Denault, S., Goldi, P., Thompson, W. G., Shuster, J., Freedman, S. O.: The detection of alpha$_1$-fetoprotein in patients with viral hepatitis. Cancer Res. **34,** 244–247 (1974).
44. Tabor, E., Gerety, R. J., Hoofnagle, J. H., Barker, L. F.: Immune response in fulminant viral hepatitis, type B. Gastroenterology **71,** 635–640 (1976).
45. Taketa, K., Watanabe, A., Kosaka, K.: Different mechanisms of increased alpha-fetoprotein production in rats following CCl_4 intoxication and partial hepatectomy. Ann. N. Y. Acad. Sci. **259,** 80–84 (1975).
46. Tatarinov, Y. S.: Detection of embryospecific alphaglobulin in the blood sera of patients with primary liver tumor. Vop. Med. Khim. **10,** 90–91 (1964).
47. Tsung, S. H.: Alpha-fetoprotein in lung cancer metastatic to the liver. Arch. Path. **99,** 267–269 (1975).
48. U. K. Collaborative study on alpha-fetoprotein: Maternal serum alpha-fetoprotein measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Lancet **1,** 1323–1332 (1977).

49. Uriel, J., Aussel, C., Bouillob, D., Loisillier, F., deNechaud, B.: Liver differentiation and the estrogen-binding proteins of alpha-fetoprotein. Ann. N. Y. Acad. Sci. **259,** 119–130 (1975).
50. Vogel, C. L., Primack, A., McIntire, K. R., Carbone, P. P., Anthony, P. P.: Serum alpha-fetoprotein in 184 Ugandan patients with hepatocellular carcinoma. Cancer **33,** 959–964 (1971).
51. Waldman, T. A., McIntire, K. R.: The use of radioimmuno-assay for alpha-fetoprotein in the diagnosis of malignancy. Cancer **34,** 1510–1515 (1974).
52. Watanabe, A., Miyazaki, M., Taketa, K.: Mechanisms of increased $alpha_1$-fetoprotein prodution in hepatic injury. Experientia (Basel) **32,** 470–471 (1976).
53. Zeltner, P. M., Neerhout, R. C., Fonkalsrud, E. W., Stiehm, E. R.: Differentiation between neonatal hepatitis and biliary atresia by measuring serum alpha-fetoprotein. Lancet **1,** 373–375 (1974).

Acta Medica Hungarica 40 (2—3), pp. 165—174 (1983)

Cardiology

LITHIUM CARBONATE IN THE MANAGEMENT OF INTRACTABLE CONGESTIVE HEART FAILURE

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(Received May 4, 1982)

Lithium carbonate was administered to 11 patients with advanced congestive heart failure resistant to the usual forms of therapy. Abundant diuresis, consecutive regression of oedema, loss of weight and clinical improvement ensued in response to lithium therapy. Doses ensuring a serum level of 0.5 to 1.0 mmol/l are regarded as optimal in heart disease. Within this range lithium produces no clinical or ECG signs of cardiotoxicity, provided the serum Na and K levels remain normal. The side-effects observed are reported in detail and attention is drawn to the necessity of follow-up and especially of checking the serum lithium level.

Keywords: heart failure; lithium carbonate therapy;

Introduction

Thyroidectomy was proposed for intractable congestive heart failure by Blumgart et al. [5] in order to reduce the O_2 demand of the body. The method, not only found favourable acceptance [31] but successful attempts with thiourea [32] and radioiodine [6] were also reported.

Cade [10] was the first to use lithium salts in psychiatry. Certain side-effects (polyuria, dehydration) were already noted by this author. In the course of subsequent observations a syndrome reminiscent of diabetes insipidus and also hypothyroidism were pointed out as hazards of the long-term use of lithium [15, 41].

We have attempted to make use of the diuretic and metabolic depressant properties of lithium in intractable congestive heart failure.

Patients and methods

A group of 11 patients, 4 males and 7 females, aged 35–85 (58 $\pm$ 11.6) years, with heart disease of major severity was studied. The therapeutic measures applied are seen in Table I; they were basically consistent with current trends [29]. When this therapy had failed (control period), the patients were started on lithium carbonate in oral doses of 300 mg t.i.d.

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Table I

*General measures and drug treatment in congestive heart failure of major severity**

1. *Physical rest — psychic relaxation*
   Bed rest + meprobamate
2. *Diet*
   5–6 g NaCl, 1000—1600 Cal
3. *Oxygen*
   Nasal tube 4—6 l/min
   Nasal tube 2 l/min (No. 10)
   IPPB (Bird M 8) (No. 11)
4. *Antihypertensive therapy*
   Alpha-methyldopa + dihydralazine (Nos 9, 10)
5. *Correction of metabolic disorders*
   Control of diabetes with diet (Nos 7, 8, 11),
   Control of diabetes with insulin (Nos 1, 9),
   4.2% $NaHCO_3$ i.v. (in individual cases)
   Hypokalaemia: oral or i.v. KCl (in individual cases)
   Hypalbuminaemia: human albumin
6. *Anticoagulant therapy*
   Acenocoumarol (Nos 1, 4, 5, 7, 8),
   Heparin 3-times 5000 U.s.c.
7. *Digitalis glycosides*
   Lanatoside-C or strophantoside-K i.v.
8. *Diuretics*
   Spironolactone + saluretics (furosemide, ethacrynic acid, clopamide), aminophylline, mannitol 20%,
   Human albumin 20%
9. *Oral vasodilators*
   Pentaerythryl-tetranitrate or isosorbide-dinitrate,
   Nitroglycerin if required
10. *Antiarrhythmic therapy*
    In individual cases 200 mg lidocaine + +30 mmol KCl + 100 mmol $NaHCO_3$
11. *Positive inotropic drugs*
    Ephedrine + atropine

* Whenever the number of the patient is not given and the note "in individual cases" is absent, the measures apply to all patients.
IPPB = intermittent positive pressure breathing

In later course the doses were adjusted individually according to the serum lithium level, which we sought to keep within the desirable range of 0.5 to 1.0 mmol/l.

The serum lithium level was checked (using a Zeiss III flame photometer) every second day during the first week, every third day during the second week, and weekly in the later course. The doses were administered in two portions daily. Blood samples were withdrawn 12 to 14 h after the last dose. Inpatients were checked weekly for serum Na, K, carbamide-N (BUN), blood glucose, bilirubin, alkaline phosphatase, creatinine, Ca and P by Technicon Autoanalyzer, serum Cl by Galenopharm Cl-rapid test, blood gases by Radelkisz OP 210/2 apparatus, and leucocyte counts. In outpatients the same tests were done at 14 day intervals.

Results

Table II shows the data of the patients and the therapy. The longest treatment lasted for 187, the shortest for 9 days. In the euthyroid cases the serum lithium level between 0.5 and 1.0 mmol/l was attained by the end of the 3rd or 4th day of treatment. After the 4th day the dose had to be reduced. As soon as the therapeutic level was attained, profuse diuresis set in which resulted in a regression of oedema and thus in clinical improvement. In the hyperthyroid patient (No. 10) it took 6 days to attain the therapeutic lithium level. Regression of oedema and subsequent control of thyrotoxicosis were of marked benefit to the circulation.

As a result of a striking improvement, 4 patients could be discharged

and then treated as outpatients. Five of the 11 patients died, 2 of them are still during hospitalization (Nos 1 and 2) and 3 after discharge (Nos 3, 4, 7). The cause of death was rapidly progressing congestive heart failure after withdrawal of lithium (Nos 1 to 4), and recurrence of myocardial infarction in one case.

Signs of lithium overdosage were noted in 7 cases. Vomiting, abdominal discomfort, drowsiness were reported by 5 patients, ECG abnormalities (ST-depression, flattening of T-waves were found at toxic lithium levels in all 7 cases, whereas at therapeutic levels these signs were indistinct, except in one single case (No. 10) where the ECG abnormalities persisted during maintenance treatment with lithium until the control of hypokalaemia. In 3 cases permature ventricular contractions, in one case deterioration of an earlier Parkinsonian syndrome gave cause for concern. In the last case (No. 9) grave dehydration and apathy ensued owing to a lack of supervision of lithium treatment.

Patient No. 11 developed CO_2 coma during the first course of lithium. During the second course his cardio-respiratory condition remained adequate so long as the lithium levels could be kept within the range of 0.4 to 0.6 mmol/l. Beyond 0.7 mmol/l deterioration of hypoxia and hypercapnia ensued without, however, causing any discomfort.

The essential laboratory findings are seen in Table III. It can be seen that during maintenance therapy with lithium (line 2 in the therapy column) all patients experienced a loss of weight and a fall in the BUN value. In 10 cases oxygenation also improved. At therapeutic lithium levels no hyponatraemia appeared. Hypokalaemia occurred in one case (No. 9) during transitory interruption of spironolactone administration.

Overdosage of lithium was associated with a deterioration of oxygenation in all 7 cases (line 3 in the therapy column). In 4 of these cases absolute CO_2 retention or relative one (disproportionate with the changes in pO_2) was found. A case of CO_2 coma has already been mentioned. These blood gase changes caused no symptoms, at least no deterioration of dyspnoea and or a further involvement of the auxiliary respiratory musculature.

At toxic lithium levels BUN increased in 3 of the 7 cases, hypokalaemia and hyponatraemia occurred in 3 cases each. The premature contractions noted in some of the cases seemed to be related to the severity of hypoxia rather than to the serum lithium level.

Discussion

Attempts at reducing the O_2 demand in intractable heart failure seemed to be promising, but the methods recommended for this purpose failed to gain ground. Thyroidectomy in patients with advanced heart failure carried a high surgical mortality [31], in addition to the hazards of consecutive tetany and

Table II

Patients and main features

No.	Age, sex	Primary disease	Duration of lithium treatment days	Observation after lithium days	Follow-up	Peak lithium level mmol/l	Side-effect	Note
1	57 ♀	Combined mitral valvular disease, diabetes, I N	9	14	0	1.4	Vomiting, ES	Died
2	47 ♂	Combined mitral valvular disease	20	56	0	1.37	Hypoxia, abdominal discomfort, drowsiness	Died
3	57 ♀	Combined mitral valvular disease	67	17	+	1.27	Vomiting, abdominal discomfort, drowsiness	Discharged, Li discontnd., died
4	62 ♀	Combined mitral valvular disease	94	15	0	1.42	Vomiting, hypoxia ES, drowsiness	Discharged, Li discontnd., died
5	58 ♀	Prior commissurotomy, Restenosis	187	69	+	1.7	Hypoxia, ES, drowsiness	Outpatient, alive
6	59 ♀	Combined mitral valvular disease	68	At present	+	0.92	Ø	Outpatient, alive
7	59 ♀	Recurrent A.M.I. diabetes, D	81	—	+	0.87	Ø	After discharge recurrence of A.M.I., died
8	57 ♂	Recurrent A.M.I., diabetes, D	123	120	+	0.92	Ø	Outpatient, compensated, alive

9	82 ♂	Coronary disease, diabetes, IN	42	187	±	1.8	Apathy, vomiting, dehydration, Parkinson syndrome	Outpatient, compensated, alive
10	71 ♀	Chronic cor pulmonale, thyrotoxicosis	63	At present	+	0.96	Ø	Outpatient, compensated, alive
11	35 ♂	Kyphoscoliotic heart disease, steroid-diabetes	42 67	20 At present	+ +	0.94 0.87	CO_2 — coma CO_2 — precoma	Outpatient Outpatient, alive

A.M.I. = acute myocardial infarction, ES = extrasystole, Diabetes, IN = insulin-controlled diabetes, Diabetes, D = diet-controlled diabetes.

In the follow-up column: 0 = no follow-up, ± = attended follow-up irregularly, + = followed up closely

Table III

Laboratory findings in the course of lithium therapy

Patient No. sex	Tb	Se.Li	Se.Na	Se.K	BUN	pO_2	pCO_2	BW kg
		mmol/l				Hg mm		
1	0	0.0	120	4.6	7.1	58	32	54
♀	1	0.0	125	3.8	10.0	56	27	56
	3	1.4	139	5.6	8.2	51	29	51
	4	0.0	125	3.8	10.7	41	22	59
2	0	0.0	141	4.2	10.7	57	37	56
♂	1	0.0	142	4.9	9.6	58	40	58
	3	1.34	139	5.7	6.6	42	35	54
	2	0.9	137	5.2	7.1	65	31	48
	4	0.0	135	4.4	17.1	44	28	60
3	0	0.0	140	4.4	8.6	54	32	43
♀	1	0.0	145	4.1	11.8	52	30	44
	3	1.65	140	4.4	8.6	40	39	40
	2	0.6	140	5.3	6.8	60	40	38
	4	0.0	129	3.6	14.3	44	31	47
4	0	0.0	134	4.7	9.4	54	30	59
♀	1	0.0	131	5.1	13.9	51	27	62
	2	0.9	140	4.9	7.5	62	34	53
	3	1.3	127	4.1	6.8	49	35	54
	4	0.0	131	4.9	17.1	41	24	64
5	0	0.0	138	3.4	10.5	52	39	59
♀	1	0.0	141	4.7	15.0	52	37	59
	2	0.6	140	4.8	10.5	66	42	53
	3	1.7	139	4.0	15.6	44	49	54
6	0	0.0	144	5.0	9.3	53	26	63
♀	1	0.0	144	4.6	6.7	55	32	66
	2	0.75	145	4.5	6.4	66	35	54
7	0	0.0	139	4.3	6.6	51	31	84
♀	1	0.0	142	3.7	7.9	53	33	84
	2	0.58	150	5.0	5.7	60	37	73
	4	0.62	139	4.1	13.1	43	19	74
8	0	0.0	146	4.7	6.0	57	35	87
♂	1	0.0	145	5.1	9.2	58	33	89
	2	0.72	140	4.9	6.1	68	38	80
9	0	0.0	142	4.4	6.8	60	44	83
♂	1	0.0	132	5.2	11.0	54	35	88
	2	1.27	143	5.1	7.2	62	38	80
	3	1.8	131	5.7	13.6	53	40	72
10	0	0.0	149	4.4	5.8	54	53	63
♀	1	0.0	147	4.5	5.2	54	48	65
	2	0.7	149	3.3	5.8	64	45	58
11	0	0.0	137	4.4	6.1	49	66	70
♂	1	0.0	149	5.4	5.4	46	78	72
	2	0.51	141	4.9	4.6	57	59	68
	3	0.94	132	3.6	13.2	41	105	65

Th = therapy, 0 = beginning of control period, 1 = end of control period, 2 = in the course of maintenance therapy, 3 = at the time of toxic manifestations, 4 = before death, Se.Li = se-lithium, Se.Na = se-natrium, Se.K = se-potassium, B U N = urea-N, BW = body-weight

myxoedema. Radioiodine acts slowly, moreover, it is difficult to keep its effect under control [7]; even if it is applied in small doses [8, 21, 22, 38] the consecutive hypothyroidism may be permanent. Thiourea derivatives have to be administered to euthyroid heart patients in massive doses for long periods, which again is a source of side-effects [32].

Our attempts at a successful management of intractable congestive heart failure prompted a search for some therapeutic factor the effect of which could be kept under control and the possible side-effects of which would be reversible after withdrawal of therapy. The diuretic effect of lithium salts has been known since their first application [10]. Later, symptoms reminiscent of diabetes insipidus were reported to be caused by lithium salts [16, 17, 35, 37, 41, 61]. Lithium is not only capable of bringing thyrotoxicosis under control [4, 24, 25, 33, 34, 45, 49] but it has also been found to produce hypothyroidism in a number of cases [15, 26, 47]. It was from these properties of lithium salts that we intended to make use in congestive heart failure.

Abundant diuresis, loss of weight, partial or complete regression of oedemas ensued in response to lithium therapy in all of our cases. In the course of maintenance therapy the initial diuresis was not followed by fluid retention [2, 18, 30, 55, 43]. It is believed that the lithium-induced reduction in body fluid space activates counter-regulatory mechanisms [2, 11] which are put out of action by the drug combination applied by us. In this sense the spironolactone-lithium combination represents a therapy of oedema and it also attenuates the side-effects of lithium by preventing K loss [5, 50].

At lithium levels of 0.5 to 1.0 mmol/l improvement of oxygenation was found. On the other hand, in case of overdosage the fall in pO_2 was not accompanied by an aggravation of dyspnoea; patient No. 11 even developed a CO_2 coma. Still, in this case, too, lithium was of benefit so long as its level remained within the range of 0.4 to 0.6 mmol/l.

Certain minor ECG abnormalities were reported to be inseparable from lithium therapy [12, 13, 28, 36, 37]. Signs of this kind were indistinct in our material at therapeutic levels of 0.5 to 1.0 mmol/l. In the case of hypokalaemia or at toxic doses [19, 27, 30, 43] the abnormalities became distinct. No conduction disturbances occurred in any of the patients [3, 14, 20, 53, 54]. Ventricular extrasystole was observed only in association with other signs of lithium intoxication or with hypoxia. Ectopic impulses may occur at toxic lithium levels [44] or with the simultaneous use of cardiotoxic arrhythmogenic drugs [1, 27]. In patient No. 6 it was during the administration of lithium, parallel with the improvement of oxygenation, that the ventricular extrasystole ceased.

Lithium has been alleged to cause cardiomyopathy or congestive heart failure [32, 42, 48]. In contrast in the present material congestive heart failure was found to improve under the effect of lithium. But then it is questionable whether lithium is in fact cardiomyopathogenic [1, 3, 46].

Five of the 11 patients had diabetes. In none of them was the carbohydrate balance affected, even in the presence of lithium intoxication [52]. The Parkinsonian manifestations deteriorated in a patient who had this syndrome, and his earlier kinetics was not restored until complete withdrawal of lithium [27, 39].

The present observations may be summed up as follows.

1. In heart failure of major severity the desirable lithium level was attained by the 3rd to 4th day after the start of treatment. Then the onset of profuse diuresis resulted in regression of oedema, loss of body weight and clinical improvement.

2. In patients with heart disease a narrower therapeutic range of serum lithium (0.5 to 1.0 mmol/l) is required than that used in current psychiatric practice, since levels above 1.2 mmol/l carry the hazard of side-effects. Lithium therapy in cor pulmonale calls for particular caution; in these cases the lithium level should not exceed 0.7 mmol/l.

3. At therapeutic dose levels no cardiotoxic effects have been noted. Certain ECG abnormalities, regarded as typical of this therapy, remained indistinct.

4. Toxic effects of lithium may be intensified in the presence of hypokalaemia or hyponatraemia. The simultaneous use of spironolactone has been found of benefit by preventing complications.

5. The cause of death was heart failure consequent upon withdrawal of lithium in four cases, and a recurrent myocardial infarction in one case.

REFERENCES

1. Albrecht, J. W., Müller-Oerlinghausen, B.: Cardiovascular side-effects of lithium. In: Johnson, F. N. (ed.): Handbook of Ltihium Therapy. MTP Press Ltd., Lancaster 1980., pp. 323–337.
2. Aronoff, M. S., Evens, R. G., Durell, J.: Effect of lithium salts on electrolyte metabolism. J. Psychiat. Res. **8**, 139–159 (1971).
3. Azar, I., Turndorf, H.: Paroxysmal left bundle branch block during nitrous oxide anesthesia in patient on lithium carbonate: a case report. Anesth. Analg. Curr. Res. **56**, 868–870 (1977).
4. Balázs, Cs., Szerze, P., Sztojka, I., Leövey, A.: A thyreotoxicosis lithium-kezelése. Magy. Belorv. Arch. **14**, 111 (1978).
5. Blumgart, H. L., Levine, S. A., Berlin, D. D.: Congestive heart failure and angina pectoris: The therapeutic effect of thyroidectomy on patients without clinical or pathologic evidence of thyroid toxicity. Arch Intern. Med. **51** 866–877 (1933).
6. Blumgart, H. L., Freedberg, A. S., Kurland, G. S.: Hypothyroidism produced by radioactive iodine (I^{131}) in the treatment of euthyroid patients with angina pectoris and congestive heart failure. Circulation **1**, 1105–1141 (1950).
7. Blumgart, H. L., Freedberg, A. S.: Conner memorial lecture: The heart and the thyroid, with particular reference to I^{131} treatment of heart disease. Circulation **6**, 222–237 (1952).
8. Blumgart, H. L., Freedberg, A. S., Kurland, G. S.: Treatment of incapacitated euthyroid cardiac patients with radioactive iodine: Summary of results in treatment of 1070 patients with angina pectoris or congestive failure. J. Amer. Med. Ass. **157**, 1–4 (1955).
9. Brown, W. T.: The pattern of lithium side-effects and toxic reactions in the course of lithium therapy. In: Johson, F. N. (ed.): Handbook of Lithium Therapy, MTP Press Ltd., Lancaster 1980., pp. 279–288.

10. Cade, J. F. J.: Lithium salts in the treatment of psychotic excitement. Med. J. Aust. **36,** 349–352 (1949).
11. Davis, J. D.: The pathogenesis of peripheral cardiac oedema. In: Bahlaman, J., Brod, J. (eds): Disturbances of Water and Electrolyte Metabolism. Karger, Basel 1980., pp. 68–74.
12. Demers, R. G., Heninger, G.: Electrocardiographic changes during lithium therapy. Dis. Nerv. Syst. **31,** 674–679 (1970).
13. Demers, R. G., Heninger, G.: Electrocardiographic T-wave changes during lithium carbonate treatment. J. Amer. Med. Ass. **218,** 381–386 (1971).
14. Eliasen, P., Andersen, M.: Sinoatrial block during lithium treatment. Eur. J. Cardiol. **3,** 308–311 (1975).
15. Emerson, C. H., Dyson, W. L., Utiger, R. D.: Serum thyrotropin and thyroxine concentrations in patients receiving lithium carbonate. J. Clin. Endocr. Metab. **36,** 338–346 (1973).
16. Forrest, J. N., Cohen, A. D., Toretti, J., Himmelhoch, J. M., Epstein, F. M.: On the mechanism of lithium-induced diabetes insipidus in man and rat. J. Clin. Invest. **53,** 1115–1123 (1974).
17. Hochman, S., Gutman, Y.: ADH antagonism and ADH independent action in rats with diabetes insipidus. Eur. J. Pharmacol. **28,** 100–107 (1974).
18. Hullin, R. P., Swinscoe, J. C., McDonald, R., Dransfield, G. A.: Metabolic balance studies on the effect of lithium salts in manic-depressive psychosis. Brit. J. Psychiat. **114,** 1561–1573 (1968).
19. Hurting, H., Dyson, W.: Lithium toxicity enhanced by diuretics. New Engl. J. Med. **290,** 748–749 (1974).
20. Jaffe, C. M.: First-degree atriventricular block during lithium carbonate treatment. Amer. J. Psychiat. **134,** 88–89 (1977).
21. Jaffe, H. L.: Multiple small dose radioiodine technic for treatment of severe cardiac disease in euthyroid patients. Ann. West. Med. Surg. **5,** 916–919 (1951).
22. Jaffe, H. L., Rosenfield, M. H., Pobirs, F. W., Stuppy, L. J.: Radioiodine treatment of euthyroid cardiac disease: four years of experience with 231 patients. J. Amer. Med. Ass. **159,** 434–439 (1955).
23. Kleinert, M.: Myokardiopathie unter Lithium-Therapie. Med. Klin. **69,** 494–499 (1974).
24. Kristensen, O., Andersen, A. H., Pallisgaard, G.: Lithium carbonate in the treatment of thyrotoxicosis. A controlled trial. Lancet **i,** 603–605 (1976).
25. Leövey, A.: Heveny endokrin krízisek. Magy. Belorv. Arch. **15,** 145–153 (1979).
26. Lindsted, G., Nilsson, L. A., Walinder, J., Skott, A., Öhman, R.: On the prevalence, diagnosis and management of lithium-induced hypothyroidism in psychiatric patients. Brit. J. Psychiat. **130,** 452–458 (1977).
27. Mann, J., Gershon, S.: Absolute and relative contra-indications to lithium treatment. In: Johnson, F. N. (ed.): Handbook of Lithium Therapy, MTP Press Ltd, Lancaster 1980, pp. 265–278.
28. McKusick, V. A.: The effect of lithium on the electrocardiogram of animals and relation of this effect to the ratio of the intracellular and extracellular concentrations of potassium. J. Clin. Invest. **33,** 598–610 (1954).
29. Morgan, D. Z., Heltene, C. E.: Congestive heart failure. In: Conn, H. F. (ed.): Current Therapy 1979. Saunders, Philadelphia 1979, pp. 201–205.
30. Orsz. Ideg- és Elmegyógyászati Intézet: Lithium-kezelés az elmeorvoslásban. Módszertani levél. Orv. Hetil. **121,** 2591–2592 (1980).
31. Parsons, W. H., Purks, W. K.: Total thyroidectomy for heart disease. Ann. Surg. **105,** 722–728 (1937).
32. Raab, W.: Thiouracil treatment of angina pectoris. Rational and results. J: Amer. Med. Ass. **128,** 249–256 (1945).
33. Réthly, E., Tremmel, O., Pálfy, A., Hikisch, M.: A hyperthyreosis lithium terápiája. Magy. Belorv. Arch. **29,** 35–38 (1976).
34. Rosser, R.: Thyrotoxicosis and lithium. Brit. J. Psychiat. **128,** 61–66 (1976).
35. Saran, B. M., Russel, G. F. M.: The effects of administering lithium carbonate on the balance of Na, K and water in manic depressive patients. Psychol. Med. **6,** 381–392 (1976).
36. Schou, M.: Electrocardiographic changes during treatment with lithium and with drugs of the imipramine-type. Acta Psychiat. Scand. S. **38,** 331–336 (1962).
37. Schou, M., Baastrup, P. C., Grof, P., Weiss, P., Angst, J.: Pharmacological and clinical problems of lithium prophylaxis. Brit. J. Psychiat. **116,** 615–619 (1970).
38. Segal, R. L., Silver, S., Yohalem, S. B., Newburger, R. A.: Use of radioactive iodine in the treatment of angina pectoris. Amer. J. Cardiol. **1,** 671–681 (1958).

39. Shopsin, B., Johnson, G., Gershon, S.: Neurotoxicity with lithium: differential drug responsiveness. Int. Pharmacopsychiat. **5,** 170–182 (1971).
40. Shopsin, B., Temple, H., Ingwer, M., Kane, S., Hirsch, J.: Sudden death during lithium carbonate maintenance. Proc. Int. Lithium Conference, New York 1978. Cit.: (1)
41. Singer, I., Rotenberg, D., Puschett, J. B.: Lithium-induced nephrogenic diabetes insipidus: in vivo and in vitro studies. J. Clin. Invest. **51,** 1081–1091 (1972).
42. Swedberg, K., Windblad, B.: Heart failure as complication of lithium treatment. Acta Med. Scand. **196,** 279–280 (1974).
43. Szücs, R.: Litiumsók pszichiátriai alkalmazása. Orvosképzés **53,** 276–279 (1978).
44. Tangedahl, T. N., Gau, G. T.: Myocardial irritability associated with lithium carbonate therapy. New Engl. J. Med. **287,** 867–869 (1972).
45. Temple, R., Berman, M., Robbins, J., Wolff, J.: The use of lithium in the treatment of thyrotoxicosis. J. Clin. Invest. **51,** 2746–2756 (1972).
46. Tilkian, A. G., Schroeder, J. S., Kao, J., Hultgren, H.: Effect of lithium on cardiovascular performance: Report on extended ambulatory monitoring and exercise testing before and during lithium therapy. Amer. J. Cardiol. **38,** 701–708 (1976).
47. Transbol, I., Christiansen, C., Baastrup, P. C.: Endocrine effects of lithium. I. Hypothyroidism, its prevalence in long-term treated patients. Acta Endocr. (Kbh.) **87,** 759–767 (1978).
48. Tseng, H. L.: Interstitial myocarditis probably related to lithium carbonate intoxication. Arch. Path. **92,** 444–446 (1971).
49. Turner, J. G., Brownlie, B. E. W., Sadler, W. A., Jensen, C. H.: An evaluation of lithium as an adjunct to carbimazole treatment in acute thyrotoxicosis. Acta Endocr. (kbh.) **83,** 86–92 (1976).
50. Vacaflor, L.: Lithium side effects and toxicity. The clinical picture. In: Johnson, F. N. (ed.): Lithium Research and Therapy. MTP Press Ltd., Lancaster 1975., pp. 211–226.
51. Vestergaard, P., Amidsen, A., Hansen, H. E., Schou, M.: Renal function of lithium treated patients. In: Schou, M., Strömgren, E. (eds): Origin, Prevention and Treatment of Affective Disorders. Academic Press, London 1979., pp. 107–113.
52. Waziri, R., Nelson, J.: Lithium in diabetes mellitus: a paradoxical response. J. Clin. Psychiat. **39,** 623–625 (1978).
53. Wellens, H. J., Cats, V. M., Düren, D. R.: Symptomatic sinus node abnormalities following lithium carbonate therapy. Amer. J. Med. **59,** 285–287 (1975).
54. Wilson, J. R., Kraus, E. S., Bailas, M. M., Rakita, L.: Reversible sinus-node abnormalities due to lithium carbonate therapy. New Engl. J. Med. **294,** 1223–1224 (1976).

Acta Medica Hungarica 40 (2—3), pp. 175—183 (1983)

Metabolism

EFFECT OF BLOOD LOSS ON THE PLASMA LEVEL OF WHOLE PEPTIDES AND THEIR FRACTIONS AT REST AND DURING EXERTION AND RESTITUTION

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(Received May 12, 1982)

The plasma level of peptides and their fractions was investigated in 12 male voluntary blood donors aged 19–23 years at rest, during exercise and restitution before and 1 and 24 h after blood withdrawal. In each test the subjects exercised on Monark's cycle ergometer under loads producing a heart rate acceleration up to 170/min (Physical Working Capacity — PWC 170). Blood loss produced a non-significant decrease in the plasma peptide level. During exercise the levels increased significantly both before ($P < 0.001$) as well as 1 h and 24 h after blood loss ($P < 0.025$). About one half of the increase was due to haemoconcentration. During restitution the levels were not significantly different from the initial values. Blood loss gave rise to a significant increase in the level of fractions of a molecular weight exceeding 3500 while the concentration of fractions of 1500–3500 molecular weight decreased. The changes in the peptide fractions were slightly different in the case of exercise performed after blood loss from those observed before blood loss. This applies first of all to fractions with a molecular weight below 750 whose concentration decreased during exercise performed after, and increased before, blood loss.

Keywords: blood letting, physical effort, plasma peptide concentration

Introduction

A sudden blood loss impairs the physical working capacity to an extent that depends on the amount of the lost blood [1, 3, 5, 8, 20]. This is due primarily to the decreased haemoglobin level and decreased oxygen supply by the blood [1, 3, 5]. Also important are the circulatory mechanisms of compensation for the loss of extracellular fluid [8]. The literature includes numerous reports on the adjustment of the circulatory and respiratory systems at rest and during exercise to the new conditions brought about blood loss [3, 8, 20]. On the other hand, the changes in the biochemical composition of blood resulting from exercise performed after a blood loss have received little attention [3].

There exist a number of recognized indices of physical capacity but new ones are being searched for that would make more information available. A promising development are the biochemical indices of physical working capacity. In an earlier study [18] we have shown correlation between the

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plasma peptid levels and the degree of physical capacity. In a group of competitive cyclists with high physical capacity we observed higher levels of plasma peptides at rest and a smaller increase of these levels during maximum load exercise than in a group of cyclists exhibiting lower levels of fitness.

In recent years blood peptides, not only those with hormone properties, have been found to play an important role in the control of various physiological properties [11, 14, 17, 24]. Their role in the mechanism of haemorrhagic shock is well-known [6, 12, 13]. Thus we have studied plasma peptide level at rest and during exercise after a predetermined amount of blood had been withdrawn. Another motivation to undertake the study was the fact that no research of this type was found in the literature available to us.

Material and methods

The subjects were 12 males aged 19–23 (mean age 21.6 ± 1.1) years, all voluntary blood donors who gave their consent to participate in the study after the purpose of the experiment had been explained to them. Seven of them gave blood for the second time, three for the third, and two for the fourth time. Previous blood lettings had taken place 4–6 months prior to this study. Clinical tests showed the subjects to be normal. They were admitted to the clinic on Monday and, after a period of rest, they were subjected to an initial ergometric test to determine the work load causing the heart rate to accelerate to 170/min and remain at a steady level during exercise for 10 min. To this end the subjects performed exercise on Monark's bicycle ergometer with the following work schedule: 10 N (Newtons) for 6 min, and 20 N for another 6 min. During each phase of the exercise the corresponding heart rate was determined and, using the methods of Holmgren et al. [10] and Sjőstrand [22] loads were calculated that would produce a heart rate acceleration to 85% of the maximum value (Physical Working Capacity — PWC 85%). The calculated loads ranged from 22.5 to 37.5 N (30.3 ± 4.1 N mean ± S.D.), while the work performed during ten minutes amounted to 89, 107.5 ± ± 12, 139.9 Joules.

The experiment proper took place on three consecutive days (Tuesday, Wednesday, Thursday) in the morning, two hours after a standard breakfast consisting of biscuits and unsweetened tea. Figure 1 presents graphically the schedule of the experiment. On Tuesday the subjects performed a ten-minute control exercise on Monark's cycle ergometer (Test I), on Wednesday an identical exercise 1 h after 400 ml of blood had been let (test II), and on Thursday they exercised 24 h after blood letting (test III). In the last minute of each exercise the HR was 175.1 ± 7.2 in test I, 181.8 ± 9.3 in test II and 175.4 ± 14.8/min in test III.

The haematocrit decreased from 0.47 ± 0.02 l/l to 0.45 ± 0.02 l/l one h, ($P < 0.005$) and to 0.43 ± 0.02 l/l 24 h after blood letting ($P < 0.001$). Red blood count which was 4.72 ± 0.25 T/l before bleeding fell to 4.57 ± 0.27 T/l one h ($P < 0.05$) and to 4.32 ± 0.23 T/l 24 h after bleeding ($P < 0.001$). The haemoglobin level which amounted to 9.38 ± 0.37 mmol/l before bleeding, fell to 9.13 ± 0.50 mmol/l one hour after bleeding ($P < 0.05$) and to 8.76 ± ± 0.37 mmol/l ($P < 0.001$) 24 h after bleeding.

Blood samples were taken from the cubital vein (no pressure was applied) immediately before each exercise (A), immediately after each phase of the exercise (B), and in the 30th minute of restitution (C). In addition, samples were taken to perform determinations not related to this study. Altogether during the three days of the experiment a total of 180 ml blood was additionally withdrawn. In order to determine the total peptide level, 5 ml of blood plasma obtained after centrifugation of 10 ml blood at 1050 *g* for 20 min was placed in a 2.5 × 100 cm Sephadex G-25 gel column [16]. Elution was done using 0.03 mol ammonium acetate pH 6.9. The flow rate of the eluate from the column was about 0.75 ml/min. Fractions of 5 ml were collected and their absorbance was determined at 280 nm. In this way three fractions were obtained. The first fraction contained proteins and peptides whose molecular weight exceeded 5000. The second fraction contained peptides with a molecular weight ranging from 500 to 5000. The third fraction contained peptides with molecular weight below 500 as

well as other low molecular compounds such as amino acids. The total peptide level was determined using a procedure [4] which makes use of *o*-toluidine and hypochlorite in part of the second fraction after it had been concentrated. The remaining part of that fraction was subjected to rechromatography in a two-column system, 1×50 cm each, filled with Sephadex gel G-10 [7]. Elution was effected by means of 0.03 mol ammonium acetate pH 6.9 [7]. The

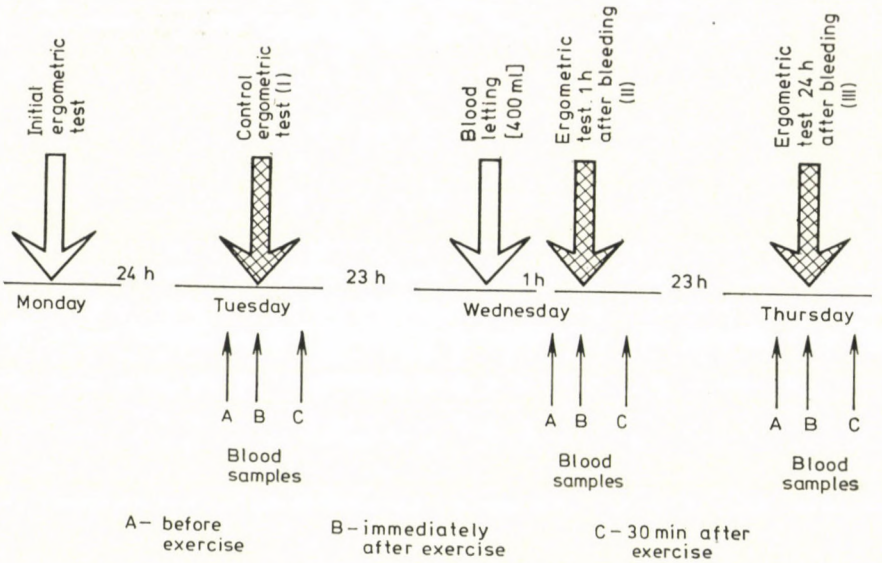


Fig. 1. Schedule of experiment in 12 voluntary blood donors who performed ten-minute bicycle ergometer exercise three times with a constant load of 30.3 ± 4.1 N (mean ± S.D.) established during the initial ergometric test

Table I

Total plasma peptide levels in 12 normal males at rest (A) immediately after exercise (B) and in the 30th minute of restitution (C) in a control test (I), 1 h after withdrawal of 400 ml blood (II) and 24 h after bleeding (III)

Group	Peptide levels mg/l		
	A	B	C
I	3.92 ± 0.59	4.55 ± 0.63 P < 0.001	4.04 ± 0.63 n.s.
II	3.79 ± 0.57	4.24 ± 0.53 P < 0.025	3.99 ± 0.60 n.s.
III	3.78 ± 0.54	4.28 ± 0.52 P < 0.025	3.92 ± 0.54 n.s.
Intergroup statistical comparison			
I/II	n.s.	n.s.	n.s.
I/III	n.s.	n.s.	n.s.
II/III	n.s.	n.s.	n.s.

Values are mean ± SD. Comparisons within groups were made by Student's paired *t* test, and between group by the unpaired *t* test; n.s. = not significant (P > 0.05)

Table II

Plasma peptide fractions of different molecular weight in 12 healthy males at rest (A), immediately withdrawal of 400 ml blood (II)

Group	Peptide fraction					
	Fraction 1 mol. wt. > 3500			Fraction 2 mol. wt. 1500—3500		
	A	B	C	A	B	C
I	13.8 ± 2.1	10.8 ± 3.2	13.7 ± 2.9	21.4 ± 1.9	20.5 ± 3.2	20.4 ± 1.9
	—	$P < 0.05$	ns	—	ns	ns
II	15.4 ± 3.7	12.6 ± 3.1	13.2 ± 4.4	19.1 ± 4.0	22.1 ± 3.7	25.7 ± 3.7
	—	$P < 0.01$	ns	—	$P < 0.005$	$P < 0.001$
III	18.5 ± 3.3	16.3 ± 3.0	16.5 ± 3.7	19.6 ± 1.3	22.8 ± 3.0	25.6 ± 2.5
	—	ns	ns	—	$P < 0.001$	$P < 0.001$
Intergroup statistical comparison						
I/II	ns	ns	ns	ns	ns	$P < 0.005$
I/III	$P < 0.005$	$P < 0.005$	ns	$P < 0.025$	ns	$P < 0.001$
II/III	ns	$P < 0.02$	ns	ns	ns	ns

Values are mean ± SD. Comparisons within groups were done by Student's paired *t*-test, and between groups by the unpaired *t* test, ns = not significant ($P > 0.05$).

flow rate of the eluate from the column was about 0.5 ml/min. Two ml fractions were then collected and their absorbance was determined at 280 nm. Four fractions were thus obtained (1, 2, 3 and 4) containing compounds which reacted positively with *o*-toluidine and hypochlorite with respect to the presence of a peptide bond. Fraction 1 contained compounds whose molecular weight exceeded 3500, fraction 2 compounds ranging from 1500 to 3500, fraction 3 compounds ranging from 750 to 1500, and fraction 4 compounds whose molecular weight was less than 750. The peptide content in each fraction was expressed in per cents of the plasma total peptide level.

Peptide levels were compared using Student's paired *t* test and differences between mean values for the groups were analysed by the unpaired *t* test. The results are presented as means ± S.D. Statistical significance was set at $P < 0.05$ [23].

Results

The plasma total peptide levels at rest, immediately after exercise and in the 30th minute of restitution are presented in Table I. Following each exercise there occurred a significant rise in the peptide level ($P < 0.001$ and $P < 0.025$, respectively). During the 30-min restitution the levels returned to the values determined at rest. No significant effect of blood loss on the peptide levels was observed at rest, immediately after the exercise, and during the restitution period, although they were highest at rest and during restitution before bleeding (Test I). Changes in the peptide levels and the haematocrit immediately after exercise and during restitution are presented in Fig. 2. It can be seen that the changes of the peptide level immediately after exercise

after exercise (B), and in the 30th minute of restitution (C) in a control test (I), one hour after and 24 h after bleeding (III)

per cent

Fraction 3 mol. wt. 750—1500			Fraction 4 mol. wt. < 750		
A	B	C	A	B	C
26.4 ± 3.1	26.8 ± 2.6	27.8 ± 1.9	38.4 ± 1.7	41.9 ± 2.2	38.1 ± 2.1
—	ns	ns	—	$P < 0.001$	ns
27.2 ± 2.7	27.7 ± 2.7	26.6 ± 2.0	38.3 ± 2.1	37.6 ± 3.7	34.5 ± 2.5
—	ns	ns	—	ns	$P < 0.005$
23.6 ± 4.9	24.3 ± 2.6	21.2 ± 1.8	38.5 ± 3.7	37.0 ± 2.8	35.7 ± 1.9
—	ns	ns	—	ns	ns
ns	ns	ns	ns	$P < 0.01$	$P < 0.005$
ns	$P < 0.05$	$P < 0.001$	ns	$P < 0.001$	$P < 0.02$
ns	$P < 0.01$	$P < 0.001$	ns	ns	$P < 0.05$

were significantly affected by the haemoconcentration, both before and after bleeding.

Table II shows the plasma peptide fractions of different molecular weights at rest, immediately after the exercise, and in the 30th minute of restitution before and after bleeding. These data show that exercise performed before blood withdrawal produced a significant decrease in the content of fraction 1 ($P < 0.05$) accompanied by a statistically significant increase in fraction 4 ($P < 0.002$). After the 30-min restitution period the changes brought about by exercise were not significantly different from those observed at rest. Exercise performed one hour after blood withdrawal produced a significant lowering of fraction 1 ($P < 0.01$) and a significant increase in fraction 2 ($P < < 0.005$). During restitution, on the other hand, there occurred a significant rise of fraction 4 ($P < 0.001$ and $P < 0.005$, respectively). The changes observed immediately after exercise and during the restitution period 24 h after blood withdrawal were similar to those observed during the test performed one hour after bleeding. There were, however, no significant changes with respect to fraction 4 as well as fractions 1 and 3. A comparison of the plasma peptide fraction content showed that immediately after the exercise as well as in the 30th minute of restitution, fraction 4 was significantly decreased ($P < 0.01$). On the other hand, bleeding produced significantly higher values for fraction 2, but only during the restitution period ($P < 0.005$). More varied in character

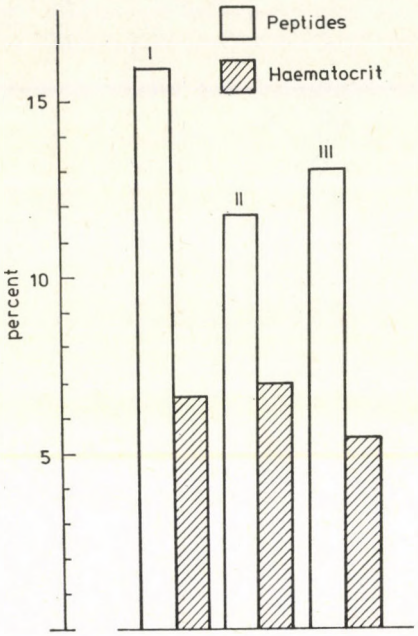


Fig. 2. Rise in plasma total peptide concentration (□) and haematocrit (▨) after physical exercise (B) in relation to the resting values (A) before blood letting (I) and 1 h (II) and 24 h (III) after blood letting. The rise is expressed in per cents of the resting value

were the changes occurring 24 h after blood withdrawal in the values obtained immediately after the exercise where a significantly higher content of fraction 1 ($P < 0.005$) and a significantly lower content of fractions 3 ($P < 0.05$) and 4 ($P < 0.001$) were observed. On the other hand during the restitution period fraction 2 was significantly increased ($P < 0.001$) while fractions 3 ($P < 0.001$) and 4 ($P < 0.02$) were significantly decreased. At rest, 24 h after bleeding, a significantly higher content of fraction 1 ($P < 0.005$) and a significantly lower content of fraction 2 ($P < 0.025$) were obtained than in the control test.

Discussion

The results presented show that physical exercise performed after bleeding gives rise to an increase in the plasma total peptide concentration and the extent of the increase is similar to that observed following exercise under normal conditions. Although the highest increase following exercise was observed in the controls (without blood loss) but the differences obtained after blood withdrawal were not significant statistically. There is no doubt that in the observed increase of total plasma peptides the haemoconcentration had an important role. Following exercise the peptide levels rose by an average

of 13.7% as compared to the values obtained before exercise, and the haematocrit rose by an average of 6.4%. This indicates that the haemoconcentration was responsible for about one half of the increase in peptide concentration following exercise. The exercise-related changes were of short duration and in the 30-min restitution period the higher levels returned to normal.

The study shows that physical exercise performed after blood withdrawal brings about changes in the content of peptide fractions different in molecular weight. The changes had a character different from those observed before bleeding. The extent of changes after the exercise and also during the restitution period, depended on the time that had passed since blood withdrawal. Exercise performed one hour after bleeding brought about a statistically significant decrease in the content of peptides whose molecular weight exceeded 3500, and the decrease was accompanied by an increase in the peptides of 1500–3500 molecular weight. On the other hand, exercise performed 24 h after the bleeding produced an increase only in the 1500–3500 molecular weight peptides. During the 30 min restitution period the higher levels of 1500–3500 molecular weight peptides were found to persist in both tests performed 1 and 24 h after bleeding, while the decreased level of the peptides of a molecular weight of less than 750 occurred only at the 1 h test.

It is difficult to explain the cause of the increase in total plasma peptides as well as the behaviour of the different peptide fractions, especially that following exercise performed before and after blood withdrawal. The difficulty lies among others in the lack of reliable data about the mechanisms controlling the peptide levels. On the one hand, they depend on their inflow from the liver and other tissues and organs, and on their intracellular degradation especially in the liver and their excretion with urine [15, 17, 19]. By changing the haemodynamic conditions of circulation a blood loss may affect both peptide formation as well as their splitting and excretion.

Of considerable interest are the findings concerning the enhanced activity of proteolytic enzymes after blood loss [21]. The release of trypsinogen and chymotrypsinogen from pancreatic zymogen granules and of cathepsin and peptidase from pancreatic lysosomal granules increases directly with the decrease in perfusion of the abdominal organs [6, 12, 13]. The phenomenon may intensify intra and extracellular protein degradation and leads to an increased flow of peptides to the circulating blood, but at the same time it may enhance the degradation of low-molecular peptides. Those with a molecular weight of 500–1000 play an important part in haemorrhagic shock by lowering myocardial contractility. A rise in their plasma concentration correlates with a decrease in mean blood arterial pressure [13].

In our studies the blood loss did not lower the blood pressure, neither the abdominal flow. For that reason, this mechanism of peptide release was of little importance in our experiments. It seems that the acidosis produced

by intensive physical exercise may have impaired the lysosomes and in this way contributed to the increase in volume of the peptides in the circulating blood [2, 9].

In view of the above one could expect that exercise performed after blood withdrawal would produce a greater increase in the plasma peptide level than exercise performed under normal conditions. It should, however, be kept in mind that blood withdrawal also lowers the protein levels. Under such conditions there occurs an increased demand for amino acids required for new proteins to be synthesized. This may be the reason why exercise performed after blood withdrawal but leads to considerable shifts in the content of peptide fractions different in molecular weight.

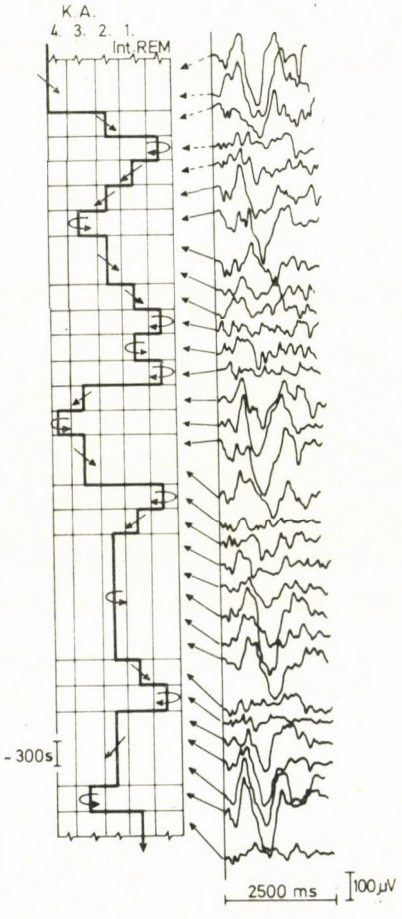
REFERENCES

1. Balke, B., Grillo, G. P., Konecci, E. B., Luft, U. C.: Work capacity after blood donation. J. Appl. Physiol. **7,** 231–236 (1954).
2. Berg, A., Keul, J.: Muscle amino acid arylopeptidases and their serum changes with exercise. Eur. J. Appl. Physiol. **36,** 267–273 (1977).
3. Bergenwald, L., Freyschuss, U., Melcher, A., Sjőstrand, T.: Circulatory and respiratory adaptation in man to acute withdrawal and reinfusion of blood. Pflügers Arch. **355,** 307–318 (1975).
4. Borel, J. P., Desanti, M., Guillaume, G., Barascut, J. L.: Une méthode de détection des peptides séparés par chromatographie sur colonne: application à l'analyse d'hydrolysats de collagène. Bull. Soc. Chim. Biol. **50,** 2165–2177 (1968).
5. Ekblom, B., Goldberg, A. N., Gullbring, B.: Response to exercise after blood loss and reinfusion. J. Appl. Physiol. **33,** 175–180 (1972).
6. Fisher, W. D., Heimbach, D. W., McArdle, C. S., Madderh, M., Hutcheson, M. M., Ledingham, I. McA.: A circulation depressant effect following canine haemorrhagic shock. Brit. J. Surg. **60,** 392–393 (1973).
7. Fürst, P., Zimmerman, L., Bergstrőm, J.: Determination of endogenous middle molecules in normal and uremic body fluids. Clin. Nephrol. 5, 178–188 (1978).
8. Gullbring, B., Holmgren, A., Sjőstrand, T., Strandell, T.: The effect of blood volume variations on the pulse rate in supine and upright positions and during exercise. Acta Physiol. Scand. **50,** 62–71 (1960).
9. Hermansen, L., Osnes, J. B.: Blood and muscle pH after maximal exercise in man. J. Appl. Physiol. **32,** 304–308 (1972).
10. Holmgren, A., Jansson, B., Levander, M., Linderholm, H., Sjőstrand, T., Strőm, G.: Low physical working capacity in suspected heart cases due to inadequate adjustment of peripheral blood flow (vasoregulatory asthenia). Acta Med. Scand. **158,** 413 (1957).
11. Leeman, S. E., Mroz, E. A., Carraway, R. E.: In: Gainer H. (ed.): Peptides in Neurobiology. Plenum Press, New York 1977., pp. 99–144.
12. Lefer, A.: Myocardial depressant factor and circulatory shock. Klin. Wschr. **52,** 358–370 (1974).
13. Lowett, W. L., Wangensteen, S. L., Glenn, T. M., Lefer, A. M.: Presence of a myocardial depressant factor in patients in circulatory shock. Surgery **70,** 223–230 (1971).
14. Lutz, W.: Isolation of a peptide inhibiting glucose absorption by red blood cells from plasma of healthy subjects performing physical exercise. Acta Physiol. Pol. **28,** 117–125 (1977).
15. Lutz, W.: A uremic peptide containing polyamine: formation and possible role in uremic hypertrigliceridemia. Physiol. Chem. Physics. **12,** 451–456 (1980).
16. Lutz, W., Markiewicz, K., Kłyszejko-Stefanowicz, L.: Oligopeptides in blood plasma of healthy humans and of patients with nephrotic syndrome. Clin. Chim. Acta, **39,** 319–326 (1972).
17. Lutz, W., Markiewicz, K., Kłyszejko-Stefanowicz, L.: Oligopeptides excreted in the urine of healthy humans and of patients with nephrotic syndrome. Clin. Chim. Acta, **39,** 425–431 (1972).

18. Markiewicz, K., Lutz, W., Cholewa, M.: The effect of maximal exertion on the levels of plasma peptides in cyclists with different physical working capacities. Eur. J. Clin. Invest. **10,** 281–284 (1980).
19. Matthews, D. M., Siamak, A. A.: Peptide absorption. Gastroenterology **71,** 151–161 (1976).
20. Robinson, B. F., Epstein, S. E., Kahler, R. L., Barunwald, E.: Circulatory effects of acute expansion of blood volume: studies during maximal exercise and at rest. Circulat. Res. **29,** 26–32 (1966).
21. Scheuch, D. W., Häcker, R., Kühne, H., Orlik, H.: Importance of enzymes in experimental and clinical shock. Z. Med. Labor. Diagn. **19,** 351–362 (1978).
22. Sjőstrand, T.: Functional capacity and exercise tolerance in patients with impaired cardiovascular function. In: Gordon Burgess, R. C. (ed.): Clinical Cardiopulmonary Physiology. Grune & Stratton, New York and London 1960.
23. Snedecor, G. W., Cochran, W. G.: Statistical Methods Applied to Experiments in Agriculture and Biology. 6th ed. Iowa State University Press, Ames, Iowa 1967.
24. Snyder, S. H., Innis, R. B.: Peptide neurotransmitters. Ann. Rev. Biochem. **48,** 755–782 (1979).

ERRATA

From the article of *Rajna, P., Halász, P., Kundra, O., Pál, I.:* "Event-related non-specific responses (K-complexes) during sleep" published in Acta Med. Hung. **40:** 33—40 (1983) Fig. 7 is missing which is presented here:



From the article of *Feledi Éva, Jobst, K.*: "Effect of Triton-100 on the electrophoretic mobility of the creatine kinase iso-enzymes of serum and tissues" published in Acta Med. Hung. **40:** 51—57 (1983) the captions of Figs 1—2 are missing which are presented here:

Fig. 1. Creatine kinase (CK) isoenzymes detected by polyacrylamide electrophoresis and by histochemical reaction with p-nitrotetrazolium blue. S_1 and S_2: two different normal sera (with 40 and 60 IU/l activity); M: skeletal muscle homogenate (with 23 000 IU/g protein activity); H: heart tissue homogenate (with 5500 IU/g protein activity); B: brain tissue homogenate (with 140 IU/g protein activity); MM, MB, BB: CK isoenzymes

Fig. 2. Creatine kinase (CK) isoenzymes detected as in Fig. 1. a and b: two different sera with elevated CK activity (680, 960 IU/l). c: heart tissue homogenate; d: brain tissue homogenate; in direction of the arrow, from top: MM, MB and BB: CK isoenzymes

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AKADÉMIAI KIADÓ

Publishing House of the Hungarian Academy of Sciences
Budapest

Distributed by KULTURA, Hungarian Foreign Trading Co., P.O.B. 149, H-1389 Budapest

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Index: 26.015

FORMERLY ACTA MEDICA ACADEMIAE SCIENTIARUM HUNGARICAE

Acta Medica Hungarica

VOLUME 40, NUMBER 4, 1983

Akadémiai Kiadó, Budapest
ACTA MED. HUNG. 40(4) 183–281 (1983) HU ISSN 0236-5286

ACTA MEDICA HUNGARICA

A QUARTERLY OF THE HUNGARIAN ACADEMY OF SCIENCES

Acta Medica publishes reviews and original papers on clinical and experimental medicine in English.

Acta Medica is published in yearly volumes of four issues by

AKADÉMIAI KIADÓ

Publishing House of the Hungarian Academy of Sciences
H-1054 Budapest, Alkotmány u. 21

Manuscripts and editorial correspondence should be addressed to the Managing Editor:

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Acta Medica is indexed in Current Contents

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Acta Medica Hungarica, Volume 40 (4), pp. 183—193 (1983)

Endocrinology

FORMATION AND CONCENTRATION OF DEHYDROEPIANDROSTERONE SULPHATE IN THE ABDOMINAL SKIN OF HEALTHY INDIVIDUALS AND PATIENTS WITH ENDOCRINE DISEASE

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(Received December 23, 1982)

In vitro incubation studies demonstrated that abdominal skin slices from healthy females and males converted 1.05–2.73% (mean 1.75%) and 0.64–2.06% (mean 1.32%), respectively of [4-^{14}C]dehydroepiandrosterone (DHA) to [4-^{14}C]dehydroepiandrosterone sulphate (DHA-S) and that the Δ^5-3β-hydroxysteroid sulphokinase(Δ^5-3β-HSS) activities were almost identical in female and male abdominal skin.

DHA-S formation in the skin of females with hirsutism of adrenocortical, ovarial or idiopathic origin was shown not to differ from that in healthy females; the hyperandrogenism in the skin of hirsute females is thus not a consequence of a Δ^5-3β-HSS deficiency. In vitro synthesis of DHA-S in the abdominal skin of patients with complete testicular feminization or 46XY pure gonad dysgenesis and anorchia was similar to that in healthy subjects.

Addition of synthetic human α^{1-39}-ACTH and natural (porcine) ACTH to in vitro incubates of abdominal skin slices from healthy females or females with various types of hirsutism increased the formation of DHA-S, revealing thereby a further extra-adrenal effect of ACTH.

In the abdominal skin of healthy females and males the DHA concentration did not differ, but the concentration of DHA-S was much higher in males.

The DHA-S concentration in the abdominal skin of idiopathic hirsutism patients was pathologically high in spite of the blood DHA-S level being normal. The considerable accumulation of DHA-S in the abdominal skin of healthy males and hirsute females cannot be explained by the normal Δ^5-3β-HSS activity; thus, it is assumed that either the DHA-S elimination mechanism may be changed in the abdominal skin slices, or the DHA-S binding proteins might be multiplied.

Keywords: metabolism of dehydroepiandrosterone, human skin tissue

Introduction

It has been known for nearly 15 years from in vitro investigations that dehydroepiandrosterone sulphate (DHA-S) is formed from dehydroepiandrosterone (DHA) in human skin [1–7]. Nevertheless, little is known of the importance of this water-soluble steroid in the skin. We have detected DHA-S in the lipoid

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film layer covering human axillary and pubic hair [5, 8]. It has also been demonstrated that apocrine sweat glands excrete a considerable quantity of DHA-S with axillary sweat [5, 9].

With a view to establishing the significance of DHA-S formation and the activity of the enzyme Δ^5-3β-hydroxysteroid sulphokinase (Δ^5—3β-HSS) in human abdominal skin, we have made a study under in vitro conditions [6] of the proportion of [4—^{14}C] DHA converted to DHA-S by abdominal skin slices from healthy females and males, and also from patients with various endocrine diseases. It was also studied how the addition of synthetic or natural ACTH to incubates of abdominal skin slices influenced the formation of DHA-S and the activity of Δ^5—3β-HSS.

In parallel, the DHA and DHA-S contents were estimated in the same abdominal skin samples from healthy females and males, and also females with hirsutism. An account of these results is presented below.

Materials and methods

Chemicals

[4-^{14}C]DHA (specific activity 57.1 μCi/mmol) and [7-^{3}H(N)] DHA (specific activity 24 Ci/mmol) were commercial products of The Radiochemical Centre (Amersham) and New England Nuclear (Boston). Before use they were purified on a 4 g Al_2O_3 column (E. Merck, Brockmann activity III/IV) and their purities were checked on an Al_2O_3—G (E. Merck) thin-layer in a TLC-G system (n-hexane — ethyl acetate — abs. ethanol — glacial acetic acid, 120 : 130 : 1 : 2, v/v/v/v/) [5]. [7-^{3}H(N)] DHA-S ammonium salt (specific activity 24 Ci/mmol) was purchased from New England Nuclear (Boston). Before use it was purified on a 1.5 g florisil column (60/100 mesh, Floridin Co., Tallahassee) [5], and its purity was then checked on silica gel G (E. Merck) thin-layer in the TLC-I system of Sarfaty and Lipsett [5, 10] (abs. ethanol — ethyl acetate — concentrated NH_4OH, 50 : 50 : 10, v/v/v) [11].

Aqueous solutions of compounds of analytical purity were prepared with glass distilled water.

The synthetic human α^{1-39}-ACTH was a product of Gedeon Richter (Budapest); 1 mg synthetic hormone corresponded to 108 I. U. [12]. The natural ACTH was strongly purified porcine ACTH (Cortrophin; Organon, Oss, The Netherlands).

The non-radioactive authentic steroids employed in the studies were procuts of Ikapharm (Ramat-Gan, Israel); before use they were purified on a 4 g Al_2O_3 column (E. Merck; Brockmann activity III/IV) and then on a thin-layer [5].

Examined subjects and skin samples

The control group of healthy males (9 cases) and females (8 cases) consisted of individuals not suffering from endocrine or other disease, who were subjected to appendectomy. The abdominal skin samples were obtained under general anaesthesia in the course of the operation.

Among the endocrine patients there were 2 cases of adrenogenital syndrome, 4 of idiopathic hirsutism, 1 of masculine ovoblastoma, 2 of complete testicular feminization, 1 of pure gonad dysgenesis and 1 of anorchia; the diagnosis was made on the basis of the clinical picture, the laboratory results and other examinations.

Skin samples from the adrenogenital syndrome patients were taken from the hirsute suprapubic abdominal area during amputation of the clitoris (subject R. A.) or during cholecystectomy (subject O. S.).

In the 4 hirsutism cases the skin samples were excised from the hirsute suprapubic abdominal area under general anaesthesia, after informed consent.

The abdominal skin samples from the complete testicular feminization, pure gonad dysgenesis and anorchia patients were obtained by lower median laparatomy in the course of diagnostic surgical exploration of the gonads.

Incubation of skin tissues

The skin samples were freed from antiseptic and adipose tissue, and cut with scissors into 1–2 mm slices. 0.5 g quantities were incubated with 0.5 μCi [4-^{14}C]DHA in 5 ml Krebs—Ringer phosphate + 200 mg/dl glucose (KRPG) medium pH 7.3 at 37 °C in air for 5 h [6]. Incubation was carried out in the presence of 10^{-3} M ATP, NAD and NADP. Bacterial contamination was inhibited by the addition of 1000 I. U. crystalline penicillin and 100 μg streptomycin sulphate per ml incubation medium [6].

Synthetic and natural ACTH were added in the same amounts (25—500 mI.U.) to the incubation medium on three occasions: at the beginning of the experiment, after 2 h, and after 4 h.

DHA-S was isolated and identified from the "sulpho-conjugated" fraction as described earlier [6]. The formed quantity of water-soluble metabolite is given in units of pmol/0.5 g wet skin tissue/5 h. The conversion was also expressed as a percentage referred to the substrate.

Determination of DHA-S concentration of serum

Direct RIA was used to determine the DHA-S in the serum of blood samples taken between 8 and 9. a.m. The antiserum was obtained by immunizing white New Zealand rabbits with DHA-17-(O-carboxymethyl)-oxime-BSA. Cross reactions were calculated according to Abraham's method and gave the following percentage values: DHA (100%), DHA-S (62.5%), Epiand (48%), Δ^4-dione (33.4%), Δ^5-diol-S (1.1%), Δ^5-diol (0.25%) and DHT (0.015%). Before the DHA-S assay the serum samples were diluted 1/1000 and 1/2000 to reduce serum steroid and protein interference [13, 14]. The results for DHA-S were expressed in μmol/l. The interassay coefficient of variation was 12.3% and 12.5% at 3.69 μmol/l and 5.99 μmol/l, respectively.

Determination of DHA and DHA-S concentrations of skin tissue

A portion (0.6–1.0 g) of the abdominal skin obtained during surgery was cut into 15–20 μ slices in a cryostat at —20 °C, and these were left to stand for 24 h in 10 ml 2 M NH_4OH solution under periodic intensive shaking. This was followed by centrifugation at 5000 r. p. m. for 10 min. The supernatant (10 ml) was removed and the residual skin tissue debris was well ground with a glass rod in 5 ml 2 M NH_4OH. After renewed centrifugation, the supernatant (5 ml) was similarly removed and combined with the former one.

The remaining skin tissue was ground with 4×5 ml methanol, and after centrifugation the methanolic extracts were combined and evaporated to dryness in vacuum. Known amounts of [7-^{3}H(N)]DHA and [7-^{3}H(N)]DHA-S were added to the dry residue, followed by the extract in 2 M NH_4OH (15 ml). The resulting solution was partially saturated by the addition of 6 g crystalline $(NH_4)_2SO_4$, the mixture was extracted with 4×15 ml ethyl acetate, and the combined extracts were evaporated to dryness in vacuum. The free and the sulphate ester steroid fractions of the extract were separated on a 1.5 g florisil column [11], and the sulphate fraction was hydrolysed with 1% $HClO_4$ in ether.

From the free androgen steroids and those liberated from the sulphate ester fraction, the DHA was isolated on a 4 g Al_2O_3 column and then purified on a thinl-ayer (E. Merck, nach Stahl) and running in a TLC-G system. Following elution from the layer, the quantites of DHA were determined by radioimmunoassay as described for serum earlier [13, 14]. The specificity of the determination was guaranteed by column and subsequent thin-layer chromatographic purification. The final results were given in units of nmol/kg of DHA or DHA-S. Results are expressed as: mean $\pm$ S.E.M.

Results

Table I shows the quantities and percentages of DHA-S formed from DHA during the incubation of abdominal skin slices from 8 healthy females (age 17–46 years) and 9 healthy males (age 19–46 years). The range of percentage

conversion of DHA to DHA-S was 1.05–2.73% for the females, and 0.64–2.06% for the males. The difference between the two groups was not significant.

The corresponding results for abdominal skin slices from the endocrine patients are listed in Table II. In all cases the DHA conversion to DHA-S lay in the range for the healthy subjects.

Table I

Formation of [4-^{14}C]DHA-S in abdominal skin slices of healthy females and males

Females					Males				
Case No.	Age years	[4-^{14}C]DHA incubated pmol	[4-^{14}C]DHA-S formed 0.5 g skin/5 h		Case No.	Age years	[4-^{14}C]DHA incubated pmol	[4-^{14}C]DHA-S formed 0.5 g skin/5 h	
			pmol	%				pmol	%
1	17	7291	127	1.74	1	19	9136	112	1.23
2	19	8187	86	1.05	2	21	8187	126	1.54
3	21	8187	117	1.42	3	21	8554	136	1.59
4	22	7291	95	1.30	4	23	8189	53	0.64
5	22	7379	202	2.73	5	30	8659	118	1.37
6	24	8554	198	2.31	6	37	9136	80	0.87
7	24	8554	196	2.29	7	39	8659	141	1.63
8	46	9136	104	1.14	8	41	8189	75	0.91
					9	46	8189	169	2.06
Limiting values			86–202	1.05–2.73				53–169	0.64–2.06
Mean			140.6	1.75				112	1.32
± S.E.M.			±17.56	±0.22				±12.23	±0.15

The effects of synthetic human α^{1-39}-ACTH and natural (porcine) ACTH on the formation of DHA-S in human abdominal skin are seen in Table III. For healthy subjects, parallel incubations without the addition of ACTH demonstrated consistent conversions (maximum difference in DHA-S formation, 6.4%). When three doses of 25–150 mI.U. synthetic ACTH were added to incubates of abdominal skin slices from three healthy females, DHA-S formation increased by 13.9–51.4%. Similar increases (17.4–64.8%) were found following three doses of 25–500 mI.U. in the cases of the hirsute patients. The porcine ACTH led to a smaller increase in the formation of DHA-S (6.7–31.6%) than did the synthetic human ACTH. It may also be seen from Table III that the extent of DHA-S formation could not be enhanced by elevating the amount of ACTH in the range applied in these experiments.

Table II

Formation of [4-^{14}C]DHA-S in abdominal skin slices of endocrine patients

Patient	Age, years	Diagnosis	State of abdominal skin	[4-^{14}C]DHA incubated pmol	[4-^{14}C]DHA-S formed 0.5 g skin/5 h.	
					pmol	%
R. A.	18	adrenogenital syndrome, hirsutism	hairy	8 964	111	1.24
O. S.	34	adrenogenital syndrome, hirsutism	hairy	8 964	165	1.84
M. I.	16	idiopathic hirsutism	hairy	9 093	155	1.70
T. Á.	16	idiopathic hirsutism	hairy	8 659	93	1.07
K. E.	17	idiopathic hirsutism	hairy	8 100	70	0.86
S. K.	23	idiopathic hirsutism	hairy	23 255	663	2.85
M. A.	45	Stein–Leventhal syndrome, hirsutism	hairy	23 255	218	0.94
K. B.	65	masculine ovoblastoma, hirsutism	hairy	8 187	169	2.06
Sz. K.	18	complete testicular feminization	hairless	8 996	133	1.48
G. I.	46	complete testicular feminization	hairless	8 996	183	2.03
N. R.	18	46 XY pure gonad dysgenesis	hairless	8 996	75	0.83
K. J.	25	anorchia	hairless	10 958	102	0.93

Table IV lists the results of DHA and DHA-S determination in the abdominal skin from healthy females and males, and females with hirsutism. The mean free DHA concentration in the abdominal skin of the 7 healthy females (age 28–46 years) was 80.3 $\pm$ 28.1 nmol/kg; the DHA-S concentration varied between limits of 26 and 345 nmol/kg, with a mean of 122.4 $\pm$ 42.8 nmol/kg.

One of the two adrenogenital syndrome patients (R. A., aged 18 years) displayed pathologically high DHA and DHA-S values: 729 and 2388 nmol/kg, respectively. The other such patient (O.S., aged 34 years) had normal free DHA and DHA-S concentrations in the abdominal skin: 205 and 136 nmol/kg, respectively. The former subject had only a short glucocorticoid pretreatment before the surgical intervention; while in the case of the latter the left adrenal has been removed at the age of 12 years, and the patient had chronic glucocorticoid therapy before excision of the skin sample.

The DHA and DHA-S concentrations were extremely high in the abdominal skin of one of the three idiopathic hirsutism patients (M. J.): 715 and 33 681 nmol/kg, respectively. In the other two patients (T. Á. and K. E.) the DHA values were normal: 66 and 114 nmol/kg, respectively; however, the concentration of DHA-S in the abdominal skin, 399 and 6893 nmol/kg were considerably in excess of the 345 nmol/kg found for healthy females.

DHA concentration in the abdominal skin of the five healthy males (aged 28–47 years) varied between 43 and 165 nmol/kg, with a mean of 96.8 $\pm$

21.4 nmol/kg. Their DHA-S concentration ranged between 977 and 3070 nmol/kg, with a mean of 1876 ± 411 nmol/kg.

The serum DHA-S concentrations of the patients are also given in Table IV. Whereas the range for healthy females was 2.7–9.9 μmol/l, with a mean of 6.4 ± 0.54 μmol/l, the DHA-S concentration in one of the adrenogenital syndrome patients (R. A.) was 13.3 μmol/l; the other patient (O. S.) had a normal DHA-S level: 5.3 μmol/l. The serum DHA-S concentration of the three idiopathic hirsutism patients lay in the range found for 20 healthy females.

Discussion

Little is known of the importance of water-soluble DHA-S formation or of its role, in human skin. Many consider DHA-S to be a biologically inactive metabolite of the weakly androgen-active DHA [5, 9]. According to this conception, the enzyme Δ^5—3β-HSS in the skin plays a part in the sulpho-conjugation and inactivation of the DHA passing into the skin from the blood stream. The free DHA reaching the skin is excreted by the sebaceous glands onto the surface of the hairs, together with sebum [5, 8, 15]. The water-soluble DHA-S is excreted onto the surface of the skin, together with the sweat, by the apocrine sweat glands [5, 9]. In another conception, the DHA-S in the skin may be transformed by sulphatase to DHA, and may become involved in the skin androgen metabolism via the pathway 4-androstene-3,17-dione → testosterone → 5α-dihydrotestosterone [16, 17]. In this way, the slowly metabolized DHA-S would be the reserve hormone of the rapidly metabolized DHA [18, 19].

Studies of abdominal skin slices from healthy females and males (Table I) have confirmed that [4—^{14}C] DHA added to the incubation medium was converted to [4—^{14}C] DHA-S and that the activity of Δ^5—3β-HSS was practically the same in the abdominal skin of healthy females and healthy males.

In the patients with adrenogenital syndrome or hirsutism of idiopathic or ovarial origin the extent of DHA-S formation did not differ from that in healthy females (Table II). This observation appears to confirm that the hyperandrogenism in the hairy abdominal skin of females with hirsutism [5, 20] is not a consequence of Δ^5—3β-HSS deficiency.

In complete testicular feminization, 46 XY pure gonad dysgenesis and anorchia, the hairless abdominal skin slices of the patients synthetized DHA-S to a similar extent as in healthy individuals.

Some caution is necessary in the evaluation of the results of experiments with synthetic human and natural (porcine) ACTH (Table III). The amounts of ACTH used were far in excess of the physiological level in the blood (11.6 pmol/l ± 0.97) [21], and can only be regarded as pharmacological quantities.

Table III

Effects of synthetic human α^{1-39}—ACTH and natural porcine ACTH on formation of DHA-S during incubation of human abdominal skin slices

Case No.	Patient	Diagnosis, sex, age	mI.U. ACTH added to 5 ml incubate	DHA-S formed 0.5 g skin/5 h	
				pmol	increase, %
1	K. E.	normal*	—	125	—
		female	—	127	1.6
		17 years	—	133	6.4
2	B. E.	normal*	—	117	—
		female	—	117	—
		21 years	3×50	137	17.1
3	D. M.	normal*	—	202	—
		female	3×50	234	15.8
		22 years	3×100	230	13.9
			3×150	231	14.3
4	S. J.	normal*	—	344	—
		female	3×25	521	51.4
		43 years			
5	S. K.	sympathectomy*	—	18	—
		male	—	20	11.1
		27 years	3×250	32	68.4
			3×500	36	89.5
6	S. K.	idiopathic hirsutism*	—	663	—
		female	3×25	1093	64.8
		23 years			
7	M. A.	Stein—Leventhal* syndrome, hirsutism	—	218	—
		female	3×25	256	17.4
8	K. B.	Masculine ovoblastoma*, hirsutism	—	169	—
		female	—	171	1.2
		65 years	3×250	199	17.0
			3×500	221	30.0
9	R. Sz.	normal**	—	95	—
		female	3×50	108	13.7
		22 years	3×100	125	31.6
10	M. F.	normal**	—	53	—
		male	—	54	1.9
		23 years	3×500	59	10.3
11	T. J.	normal**	—	60	—
		male	3×250	64	6.7
		41 years	3×500	71	18.3

* Synthetic human α^{1-39}-ACTH
** Natural porcine ACTH

ACTH was added to the incubate repeatedly during the experiment in order to replace the decomposed ACTH. Besides thermal decomposition, it is also necessary to take into consideration the adsorption of ACTH to the wall of the vessel. Accordingly, it is not surprising that large individual differences in DHA-S formation were observed and that a correlation could not be demonstrated between the amounts of ACTH added and the quantities of DHA-S. The only definite conclusion that can be drawn from the results is that both the synthetic human and the porcine ACTH enhanced the formation of DHA-S in the incubates of abdominal skin slices from both healthy and hirsute females. The phenomenon means that we have observed a hitherto unknown extra-adrenal effect of ACTH. The effect manifests with an enhancement of the activity of Δ^5—3β-HSS, which plays a part in the sulpho-conjugation of DHA reaching the skin from the blood stream. The newly demonstrated effect of ACTH needs further investigations.

It has long been assumed that the composition and quantities of androgen steroids in human skin arise from steroids passing into the skin from the blood stream and those formed locally in the skin [5]. It is known that in human skin an intensive androgen steroid metabolism occurs [23—28]. This is determined under both normal and pathological conditions by the enzyme systems of the skin, the various binding proteins and the receptor proteins.

DHA enters the abdominal skin tissue from the blood stream and, depending on the activity of the skin enzyme systems, it is metabolized in various directions [3, 6, 25, 28] including sulpho-conjugation [1, 2, 4]. The skin tissue contains more DHA than that circulating in the blood of healthy females and males: 13.98 $\pm$ 7.49 and 18.46 $\pm$ 4.65 nmol/l, respectively [29]; at the same time, the DHA-S concentration is much lower in the abdominal skin than in the blood. The DHA concentration is the same in the abdominal skin tissues of healthy females and males, whereas the DHA-S concentration is much higher in males than in females (Table IV).

In one adrenogenital syndrome patient (R. A.) where the excess androgen was produced by the diseased adrenal cortex and the DHA-S level was pathologically high in the serum, both the free DHA and the DHA-S were found to be elevated in the abdominal skin. In an other adrenogenital case (O. S.) the DHA-S concentration was normal in the serum and so were the DHA and DHA-S concentrations in the skin.

The free DHA concentration on the abdominal skin tissue was pathologically high in one of the hree idiopathic hirsutism patients, and normal in the other two. The concentration of DHA-S was, however, pathologically high in the abdominal skin of all three patients (in two it was extremely high), in spite of the blood DHA-S level having been normal in all of them.

The extensive accumulation of DHA-S in the abdominal skin of healthy males and of patients with hirsutism is not explained by the Δ^5—3β-HSS

Table IV

DHA and DHA-S concentration in abdominal skin of healthy females, healthy males and hirsute females and DHA-S concentration in serum of hirsute patients

Case, No	Patient	Age, years	Diagnosis	Abdominal skin tissue		Serum DHA-S μmol/l
				DHA, nmol/kg	DHA-S, nmol/kg	
FEMALES						
1	N. A.	28	normal	59	82	—
2	K. L.	30	normal	243	128	
3	L. L.	37	normal	39	42	
4	T. I.	27	normal	22	345	
5	Sz. J.	39	normal	52	26	
6	B. G.	42	normal	62	187	
7	F. K.	46	normal	85	47	
Limits		28–46		22–243	26–345	
Mean		37		80.3	122.4	
± S.E.M.				±28.1	±42.8	
FEMALES						
1	R. A.	18	adrenogenital syndrome, hirsutism	729	2 388	13.3
2	O. S.	34	adrenogenital syndrome, hirsutism	205	136	5.3
3	M. J.	16	idiopathic hirsutism	715	33 681	7.8
4	T. Á.	16	idiopathic hirsutism	66	399	4.5
5	K. E.	17	idiopathic hirsutism	114	6 893	7.5
MALES						
1	N. J.	28	normal	165	1422	
2	B. Z.	30	normal	70	1273	
3	L. P.	37	normal	83	3070	
4	T. P.	39	normal	43	977	
5	C. J.	47	normal	123	2638	
Limits		28–47		43–165	977–3070	
Mean		36		96.8	1876	
±S.E.M.:				±21.4	±411	

DHA-S concentration μmol/l in serum of healthy females and males

Females (20 cases)		Males (20 cases)
Limits:	2.7–9.9	5.8–12.4
Mean:	6.4	8.2
±S.E.M.:	±0.54	±0.41

activity. The DHA-S passing into the abdominal skin or formed there might have an altered elimination mechanism, but it may also be presumed that in the hairy abdominal skin of healthy males and hirsute females proteins accumulated which cause the binding of DHA-S.

REFERENCES

1. Gallegos, A. J., Berliner, D. L.: Transformation and conjugation of dehydroepiandrosterone by human skin. J. clin. Endocr. **28,** 1214–1218 (1967).
2. Faredin, I., Tóth, I., Fazekas, Á. G., Kókai, K., Julesz, M.: Conjugation in vitro of [4-^{14}C]dehydroepiandrosterone to [4-^{14}C]dehydroepiandrosterone sulphate by normal human female skin slices. J. Endocr. **41,** 295–296 (1968).
3. Berliner, D. L., Pasqualini, J. R., Gallegos, A. J.: The formation of water soluble steroids by human skin. J. invest. Derm. **50,** 220–224 (1968).
4. Hay, J. B., Hodgins, M. B.: The androgen metabolism of human skin. J. Endocr. **51,** 23–24 (1971).
5. Julesz, M., Faredin, I., Tóth, I.: Steroids in Human Skin. Akadémiai Kiadó, Budapest 1971, pp. 138–141
6. Faredin, I., Tóth, I., Julesz, M.: The metabolism of [4-^{14}C]dehydroepiandrosterone by human skin in vitro. II. In vitro formation of water soluble dehydroepiandrosterone sulphate and androst-5-ene-3β,17β-diol-3-sulphate in human abdominal skin. Acta med. Acad. Sci. hung. **30,** 91–99 (1973).
7. Hay, J. B., Hodgins, M. B.: Metabolism of androgens in vitro by human facial and axillary skin. J. Endocr. **59,** 475–486 (1973).
8. Tóth, I., Faredin, I.: Androgen steroids in human axillary hair lipids. Acta physiol. Acad Sci. hung. **51,** 209–210 (1978).
9. Tóth, I., Julesz, J., Faredin, I.: Androgen steroids in human axillary sweat. Acta physiol. Acad. Sci. hung. **52,** 269 (1978).
10. Sarfaty, G. A., Lipsett, M. B.: Separation of free and conjugated 11-deoxy-17-oxysteroids by thin-layer chromatography. Anal. Biochem **15,** 184–186 (1966).
11. Tóth, I., Faredin, I.: Separation and identification of free and esterfied 17-ketosteroids. Acta med. Acad. Sci. hung. **29,** 141–144 (1972).
12. Szporny, L., Hajós, Gy. T., Szeberényi, Sz., Fekete, Gy.: Biological activity of synthetic 1–39 human corticotropin. Nature (Lond.) **218,** 1169–1170 (1968).
13. Zappulla, F., Ventura, D., Capelli, M., Cassio, A., Balsamo, A., Fréjaville, E., Bolelli, G., Cacciari, E.: Gonadal and adrenal secretion of dehydroepiandrosterone sulfate in prepubertal and pubertal subjects. J. endocr. Invest. **4,** 197–202 (1981).
14. Peretti, E., Forest, M. G.: Radioimmunoassay method for unconjugated plasma dehydroepiandrosterone. In: Gupta, D. (ed.): Radio-Immunoassay of Steroid Hormones, 2nd ed. Verlag Chemie, Weinheim 1980, pp. 81—90.
15. Karunakaran, M. E., Pochi, P. E., Strauss, J. S., Valerio, E. A., Wotiz, H. H., Clark, S. J.: Androgens in skin surface lipids. J. invest. Derm. **60,** 121–125 (1973).
16. Kim, M. H., Hermann, W. L.: In vitro metabolism of dehydroepiandrosterone sulphate in foreskin, abdominal skin and vaginal mucosa. J. clin. Endocr. **28,** 187–191 (1968).
17. Drucker, W. D., Blumberg, J. M., Gandy, H. M., David, R. R., Verde, A. L.: Biologic active dehydroepiandrosterone sulphate in man. J. clin. Endocr. **35,** 48—54 (1972).
18. Wang, D. J., Bulbrook, R. D.: The metabolic clearence rates of dehydroepiandrosterone, testosterone and their sulphate esters in man, rat and rabbit. J. Endocr. **38,** 307–318 (1967).
19. Rosenfeld, R. L., Hellman, L., Gallagher, T. F.: Metabolism and interconversion of dehydroepiandrosterone and dehydroepiandrosterone sulphate. J. clin. Endocr. **35,** 187–193 (1972).
20. Burton, I. L., Johnson, C., Libman, L., Shuster, L.: Skin virilism in women with hirsutism. J. Endocr. **53,** 349–354 (1972).
21. Julesz, J., Janáky, T., Laczi, F., Kárteszi, M., László, F. A., Stark, E.: Plasma ACTH level and ACTH reserve in adrenocortical disease. Acta med. Acad. Sci. hung. **38,** 1—11 (1981).
22. Faredin , I., Webb, J. L., Julesz, M.: The in vitro metabolism of dehydroepiandrosterone by human skin. Acta med. Acad. Sci. hung. **23,** 169–180 (1967).
23. Faredin, I., Fazekas, Á. G., Kókai, K., Tóth, I., Julesz, M.: The in vitro metabolism of [4-^{14}C] dehydroepiandrosterone by human male pubic skin. Eur. J. Steroids **2,** 223–242 (1967).
24. Faredin, I., Fazekas, Á. G., Tóth, I., Kókai, K., Julesz, M.: Transformation in vitro of [4-^{14}C] dehydroepiandrosterone in female skin tissue. J. invest. Derm. **52,** 357–361 (1969).
25. Faredin, I., Tóth, I., Fazekas, Á. G., Kókai, K., Julesz, M.: The in vitro metabolism of [4-^{14}C]androst-4-ene-3,17-dione by normal and hirsute female skin. Int. J. Derm. **9,** 147–152 (1970).

26. Faredin, I., Tóth, I.: Biotransformation of [4-^{14}C] 4-androstene-3,17-dione by healthy human skin in vitro. Acta med. Acad. Sci. hung. **32,** 129–137 (1975).
27. Faredin, I., Tóth, I.: The metabolism of [4-^{14}C]5-androstene-3β,17β-diol by normal human skin in vitro. Acta med. Acad. Sci. hung. **32,** 139–152 (1975).
28. Faredin, I., Kókai, K., Tóth, I., Fazekas, Á. G., Julesz, M.: The metabolism of [4-^{14}C]dehydroepiandrosterone by human skin in vitro. I. Transformation in vitro of [4-^{14}C]dehydroepiandrosterone into androst-4-ene-3,17-dione, testosterone by normal human male and female skin slices. Acta med. Acad. Sci. hung. **27,** 95–102 (1970).
29. Rosenfield, R. L.: Competitive protein binding method for the measurement of unconjugated and sulphate-conjugated dehydroepiandrosterone in peripheral plasma. Steroids **17,** 689–696 (1971).

Acta Medica Hungarica, Volume 40 (4), pp. 195—202 (1983)

Immunology

SENSITIVITY OF PREINCUBATED LYMPHOCYTES TO SUPPRESSOR REGULATION

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(Received October 12, 1982)

DNA synthesis of human peripheral blood lymphocytes increases if Con A is added to the culture after 24 h preincubation at 37 °C. During preincubation the mitogen reactive lymphocytes lose their sensitivity to the suppressive effect of autologous mitomycin-treated mononuclear leukocytes, and of supernatants of autologous preincubated cells. The reactive lymphocytes preincubated at 4 °C retain their sensitivity to the suppressive effect of regulatory cells and their supernatants. It is assumed that the enhancement of mitogen response after preincubation at 37 °C is caused by a decrease of suppressor regulation of human lymphocytes. Prostaglandins may be regarded as one of the mediators of the suppression.
Keywords: DNA synthesis, lymphocytes, supressor regulation

Introduction

Suppressor cells inhibit various immune reactions. They can be induced by antigens [1, 2, 10], mitogens [5, 7, 12, 15] and by allogeneic cells [7], though spontaneous activity has also been described. Preincubation of human lymphocytes results in an increased mitogenic response, if the mitogen is used in suboptimal concentration. This has been explained by the loss of "short lived" suppressor cell activity during preincubation at 37 °C [8]. Stobo et al. (1976) found only a slight elevation of proliferation after preincubation; the reason for this might be the higher mitogen concentration used [17]. Following preincubation at 4 °C, the mitogenic response remained unchanged, therefore Feighery et al. (1978) supposed the survival of suppressor cells at 4 °C [6]. McCombs et al. (1978) demonstrated that the augmentation of response was due in part to an enhancement of lymphocyte activation [14]. Fresh cells added to the precultured cells failed to suppress the augmented response [14, 16]. Goodwin et al. [8, 9] supposed a connection between the short lived suppressor phenomenon and prostaglandin (PG) producing suppressor cells. The aim of the present work was to investigate the above relations.

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Materials and methods

Separation of cells

Heparinized venous blood was drawn from healthy volunteers not taking any drugs. Peripheral blood mononuclear leukocytes (PBMLs) were separated on Ficoll (Pharmacia) gradient [3]. The viability of cells was tested by trypan blue dye exclusion; it was always higher than 95%.

Preincubation of cells

PBML at 10^6 cells/ml were preincubated at 37 °C and 4 °C for 24 h and they were washed twice in TC-199 medium. Culture conditions were as described in lymphocyte DNA synthesis assay.

Mitomycin (MMC) treatment of "regulatory" cells

DNA synthesis of regulatory cells was inhibited by MMC treatment before Con A stimulation [10]. Freshly separated or preincubated cells were resuspended in TC-199 medium containing 25 μg/ml mitomycin-C (Sigma) and incubated at 37 °C for 1 h. Cells without MMC treatment with intact DNA synthesis were designated as "reactive" (= mitogen-reactive) cells.

Lymphocyte DNA synthesis assay

The separated PBMLs were resuspended in TC-199 medium supplemented with 10% heat-inactivated fetal calf serum (Humán, Budapest), antibiotics, and 25 mM HEPES (Serva). 2×10^5 cells in 5 replicates were placed into flat-bottomed microplates (Greiner). Con A (Calbiochem) was added to a final concentration of 2.5 or 25 μg/ml. The cultures were kept in humidified atmosphere at 37°C for 72 h.

Eight hours before harvesting, 0.5 μCi ^{3}H-thymidine (Amersham) was added to each well, the cultures were sucked off onto glass fibre filters (Whatman GF/c). Isotope determination was made in a Nuclear Chicago Isocap 300 counter and the results were expressed in c.p.m.

Cocultivation of "regulatory" and "reactive" cells

According to the procedure described above, 10^5 cells treated with MMC (= regulatory cells) and 10^5 untreated autologous (= reactive) cells were mixed. Controls containing only reactive cells were also set up. The effect of freshly separated and preincubated (at 4 °C) regulatory cells was compared to that of preincubated cells at 37 °C (= 100%).

Effect of supernatants of preincubated PBMLs on autologous lymphocyte DNA synthesis

10^6/ml PBMLs were incubated in plastic tubes at 4 °C and 37 °C for 24 h. The effect of supernatants obtained by centrifugation was tested on DNA synthesis of freshly separated and preincubated lymphocytes which were cultured in the supernatants of precultured autologous cells.

Effect of exogenous and endogenous PGs on DNA synthesis of Con A stimulated lymphocytes

The effect of exogenous PGE_1 and PGE_2 (Sigma) was tested. Endogenous PG synthesis was inhibited by 1 μg/ml indomethacin (INDO, Sigma, USA). PGE_1 and INDO were added to the cells immediately before adding Con A.

Analysis of data

Statistical significance was evaluated by Student's *t* test. Results are expressed as mean ± S.E.M.

Results

Effect of preincubation on Con A stimulated DNA synthesis of human lymphocytes

The 2.5 μg/ml Con A-induced DNA synthesis was considerably increased when the lymphocytes had been preincubated at 37 °C for 24 h (Fig. 1). The preincubated cells were washed in TC-199 and cultured in fresh culture medium to abolish the effect of supernatant factors. The DNA synthesis of 25 μg/ml Con A-stimulated lymphocytes also increased after preincubation at 37 °C, but to a smaller extent. Con A reactivity of lymphocytes did not change after incubation at 4 °C.

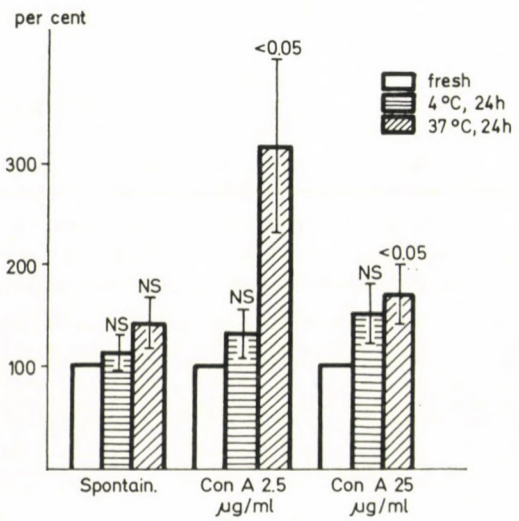

Fig. 1. Effect of preincubation at 4 °C and 37 °C on lymphocyte DNA synthesis stimulated with Con A. (Mean of 8 separate experiments, mean ± S E.M., data are expressed in per cent of control)

Effect of MMC-treated regulatory cells on DNA synthesis of freshly separated and preincubated lymphocytes

Freshly separated and preincubated (at 4 °C and 37 °C) cells after MMC treatment ("regulatory cells") were added to autologous lymphocytes stimulated with Con A ("reactive cells"). If reactive cells were preincubated at 37 °C, the effect of freshly separated and preincubated (at 37 °C and 4 °C) regulatory cells was the same (Fig. 2a). If the regulatory cells were added to freshly separated or preincubated (at 4 °C) reactive cells the suppressive effect of freshly separated and preincubated regulatory cells at 4°C was significantly greater compared to that of cells preincubated at 37 °C (Fig. 2b, c).

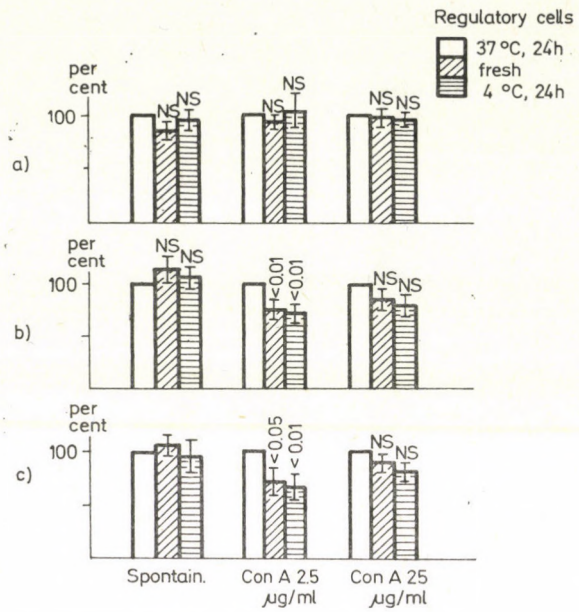

Fig. 2. Effect of freshly separated and preincubated regulatory (MMC-treated) cells on DNA synthesis of fresh and preincubated lymphocytes stimulated with Con A. (Mean of 8 separate experiments, mean ± S.E.M.; data are expressed in per cent of control.)

Effect of supernatants of preincubated cells on DNA synthesis of fresh or preincubated lymphocytes

DNA synthesis of Con A-stimulated fresh lymphocytes was inhibited by supernatants of autologous PBMLs preincubated for 24 h. Suppressive activity appeared in the supernatant of cells preincubated both at 4 °C and 37 °C (Fig. 3a). The DNA synthesis of lymphocytes preincubated at 37 °C in the presence of supernatant produced at 37 °C was higher than that of fresh lymphocytes (Fig. 3*b*). The lymphocytes preincubated at 4 °C and stimulated with 2.5 μg/ml Con A retained their sensitivity to the suppressive effect of supernatants (Fig. 3c).

Effect of PGEs on DNA synthesis of lymphocytes

The effect of PGE_1 and PGE_2 was investigated on the proliferative response at low and high doses of mitogen. The inhibitory effect of PGEs on DNA synthesis was more pronounced at a low mitogen dose (Figs 4–5). Lymphocyte proliferation was decreased by PGE_1 and PGE_2 even in very low concentration (the endogenous PG synthesis was simultaneously inhibited by INDO).

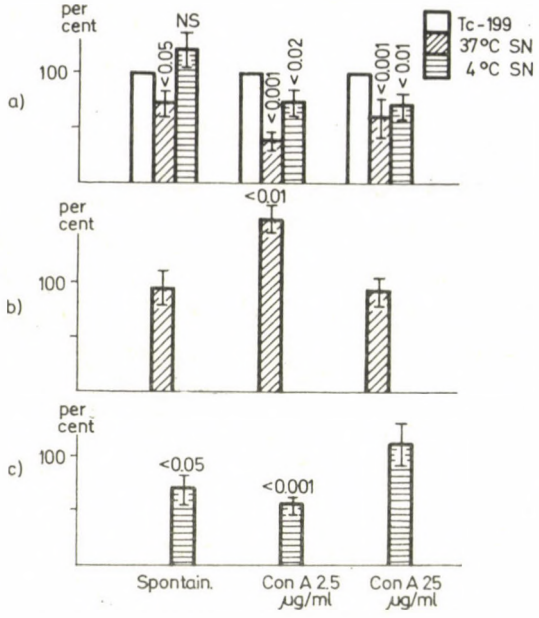

Fig. 3. Effect of supernatants (SN) produced by autologous mononuclear leukocytes preincubated at 4 °C and 37 °C for 24 h on DNA synthesis of fresh and preincubated lymphocytes. (Mean of 8 separate experiments, mean ± S.E.M.; data are expressed in per cent of control.)

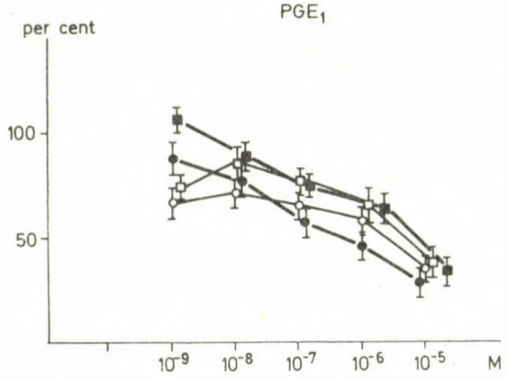

Fig. 4. Effect of PGE_1 on DNA synthesis of Con A stimulated lymphocytes. Endogenous PG production was abolished by INDO (1 µg/ml). (Mean of 9 separate experiments, mean ± S.E.M.; data are expressed in per cent of control.) ○ Con A, 2.5 µg/ml; ● Con A, 2.5 µg/ml + INDO, 1 µg/ml; □ Con A, 25 µg/ml; ■ Con A, 25 µg/ml + INDO, 1 µg/ml

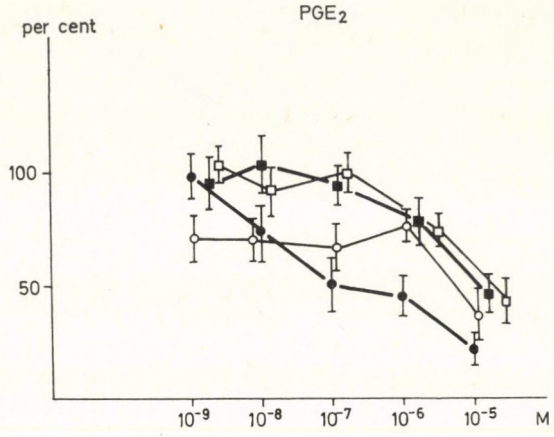

Fig. 5. Effect of PGE_2 on DNA synthesis of Con A stimulated lymphocytes. Endogenous PG production was abolished by INDO(1 μg/ml). (Mean of 6 separate experiments, mean ± S.E.M.; data are expressed in per cent of control.) ○ Con A, 2.5 μg/ml; ● Con A, 2.5 μg/ml + INDO, 1 μg/ml; □ Con A, 25 μg/ml; ■ Con A, 25 μg/ml + INDO, 1 μg/ml

Discussion

The short-lived suppressor phenomenon has been supposed to be due to the disappearance of suppressor cells during preincubation at 37 °C for 24 h, which could be shown in lymphocyte cultures using mitogen at a suboptimal concentration [4, 6]. A close connection between the short lived suppressor cell function and PG production was supposed by Goodwin et al. [8, 9]. Our aim was to study the cause of enhancement of lymphocyte proliferation after preincubation. There were two possible explanations for the elevation of DNA synthesis of preincubated lymphocytes, (*i*) an alteration of suppressor cell function; and (*ii*) a change of reactive lymphocyte sensitivity to suppression.

We found that the proliferative response to Con A, both at low and high concentrations, increased after preincubation of lymphocytes at 37 °C for 24 h. The supernatants of preincubated cells were removed and replaced by fresh culture medium. The enhancement of DNA synthesis following preincubation was substantially more marked with a low Con A dose than with a higher one (+200% and +50%, respectively). Supposing a disappearance of suppressor cells after incubation at 37 °C, we examined whether the freshly separated lymphocytes and preincubated cells at 4 °C had a greater suppressive effect than did preincubated cells at 37 °C. If the mitogen reactive lymphocytes were preincubated at 37 °C, the effect of freshly separated cells and preincubated ones at 4 °C or 37 °C was not different. If the mitogen reactive lymphocytes were freshly separated or preincubated at 4 °C, the suppressor effect of

freshly separated regulatory cells and those preincubated at 4 °C was greater compared to that of preincubated ones at 37 °C.

If the suppressive effect of regulatory cells is mediated by soluble factor(s) appearing in the supernatant of preincubated lymphocytes, the effect should change similarly as the change of the suppressor effect of regulatory cells. We found that the proliferation of freshly separated lymphocytes was inhibited by supernatants produced by autologous lymphocytes preincubated at 4 °C and 37 °C. The suppressive effect of supernatants was most striking when the supernatants were produced at 37 °C and tested on the DNA synthesis of fresh lymphocytes. The lymphocytes preincubated at 4 °C retained their sensitivity to the suppressive effect of autologous supernatants; while the cells preincubated at 37 °C failed to do so.

A marked decrease in the sensitivity of mitogen reactive lymphocytes to exogenous PGE_1 and PGE_2 was found after preincubation at 37 °C for 24 h. A similar phenomenon was observed in connection with the "endogenous" PG effect [13]. A significant suppression of DNA synthesis is caused by PGEs at such low concentration (10^{-8}–10^{-9} M) which can be found in leukocyte cultures in vitro (8), and in inflammatory sites in vivo (11). Suppression by exogenous PGE_1 and PGE_2 was higher at lower Con A concentration (2.5 μg/ml) than at a higher one. A similar relation was found between the Con A concentration and the suppressor effect of supernatants of preincubated cells.

Acknowledgement

The authors thank Mrs Julia Czvikovszky for expert technical assistance. This work was supported by the State Office for Technical Development.

REFERENCES

1. Basten, A., Miller, J. F. A. P., Sprent, J., Cheers, C.: Cell-to-cell interaction in the immune response. X. T-cell-dependent suppression in tolerant mice. J. exp. Med. **140,** 199–217 (1974).
2. Benjamin, D. C.: Evidence for specific suppression in the maintenance of immunological tolerance. J. exp. Med. **141,** 635–646 (1975).
3. Böyum, A.: Separation of leukocytes from blood and bone marrow. Scand. J. clin. Lab. Invest. 21 (Suppl.) **77** (1968).
4. Bresnihan, B., Jasin, H. E.: Suppressor function of peripheral blood mononuclear cells in normal individuals and in patient with systemic lupus erythematosus. J. clin. Invest. **59,** 106–116 (1977).
5. Dutton, R. W.: Inhibitory and stimulatory effects of Concanavalin A on the response of mouse spleen cell suspension to antigen. I. Characterisation of the inhibitory cell activity. J. exp. Med. **136,** 1445–1460 (1972).
6. Feighery, C., Whelan, C. A., Weir, D. G., Greally, J. F.: In vitro studies of suppressor cell function in human peripheral blood mononuclear cells. Clin. exp. Immunol. **32,** 459–465 (1978).
7. Folch, H., Waksman, B. H.: The splenic suppressor cell. I. Activity of thymus-dependent adherent cells: Changes with age and stress. J. Immunol. **113,** 127–139 (1974).
8. Goodwin, J. S., Bankhurst, A. D., Messner, R. P.: Suppression of human T-cell mitogenesis by prostaglandin. Existence of a prostaglandin-producing suppressor cell. J. exp. Med. **146,** 1719–1734 (1977).

9. Goodwin, J. S., Messner, R. P., Peake, G. T.: Prostaglandin suppression of mitogen-stimulated lymphocytes in vitro. Changes with mitogen dose and preincubation. J. clin. Invest. **62,** 753–760 (1978).
10. Ha, T. Y., Waksman, B. H.: Role of the thymus in tolerance. X. "Suppressor" activity of antigen-stimulated rat thymocytes transferred to normal recipients. J. Immunol. **110,** 1290–1299 (1973).
11. Higgs, G. A., Vane, J. R., Hart, F. D.: Effects of anti-inflammatory drugs on prostaglandins in rheumatoid arthritis. In: Robinson, H. J. and Vane, J. R. (eds): Prostaglandin Synthetase Inhibitors. Raven Press, New York 1974. pp. 165–173.
12. Jacobs, D. M.: Effects of Concanavalin A on the in vitro responses of mouse spleen cells to T-dependent and T-independent antigens. J. Immunol. **114,** 365–370 (1975).
13. Kalmár, K., Gergely, P.: Enhancement of mitogen-induced lymphocyte proliferation after preincubation is due to the altered sensitivity to prostaglandins. Immunol. Lett. **4,** 179–183 (1982).
14. McCombs, C. C., Michalski, J. P., Talal, N.: Acceleration of human lymphocyte activation after preliminary culture. J. Immunol. **120,** 532–537 (1978).
15. Rich, R. R., Pierce, C. W.: Biological expressions of lymphocyte activation. I. Effects of phytomitogens on antibody synthesis in vitro. J. exp. Med. **137,** 205-223 (1973).
16. Sohne, P. G., Collings-Lech, C.: Differentiation of the effects of preincubation and indomethacin on lymphocyte transformation. Clin. Immunol. Immunopath. **13,** 47–55 (1979).
17. Stobo, J. D., Paul, S., Van Scoy, R. E., Hermans, P. E.: Suppressor thymus-derived lymphocytes in fungal infection. J. Clin. Invest. **57,** 319–328 (1976).

Acta Medica Hungarica, Volume 40 (4), pp. 203—213 (1983)

Hepatology

CORRELATION BETWEEN BIOCHEMICAL TESTS, PARAMETERS OF DRUG ELIMINATION AND HEPATIC ENZYME INDUCTION IN CHRONIC LIVER DISEASES

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(Received June 18, 1982)

The biochemical liver function tests form an integral part of diagnostic practice in hepatology. In this study the parameters suited for the estimation of drug elimination and of the induced state of the liver, thus the excretion of D-glucaric acid, of menthol glucuronide and the parameters of antipyrine and sulphadimidine kinetics were correlated with the results of biochemical laboratory tests in patients with chronic liver disease. The aim was to establich universally valid quantitative correlations between the above results. Multiple relationships were revealed, but generally valid correlations for the group of liver diseases occurred only in special relationships (serum albumin level and antipyrine $T_{1/2}$; serum bilirubin level and sulphadimidine $T_{1/2}$). The results have been evaluated from the methodological aspect to work out an appropriate dosage regime in liver diseases.

Keywords: antipyrine kinetics, excretion of D-glucaric acid, human pharmacokinetics, liver disease, liver function tests, sulphadimidine kinetics.

Introduction

Nowadays the studies of certain biochemical parameters generally called "routine liver function tests" still belong to the important methods of hepatological diagnostics. These are supplemented by histological findings in liver biopsy specimens. A given biochemical syndrome generally implies typical microscopic features, though in practice a complete overlap does not occur [21, 25].

Impairment of the functional capacity of the diseased liver is reflected mainly in its abnormal biochemical pattern [9]. In a wider sense the drug metabolizing ability and the adaptation phenomenon termed "enzyme induction" are also liver functions [3, 5]. The diseased liver is generally considered to be an induced condition [1], and therefore it is uncertain whether some of its measurable functions such as D-glucaric acid excretion reflect a state of

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induction or damage in some diseases [7, 13, 14, 15]. Other functions, such as gamma-GT activity may reflect cholestasis in individual cases, but if the circumstances of induction are present, for example, the effect of alcohol, they may also reflect an induced state [20, 25]. Their evaluation raises various possibilities. Examination of the biochemical tests called "routine liver functions" has become general practice. Measurement of the parameters of drug metabolizing ability, or of induction requires special knowledge and equipment and, besides, it is inconvenient for the patient. So it is common practice to draw conclusions from the abnormalities of biochemical liver function tests to changes in drug elimination [27]. Another possibility is to use the histological findings as the basis of conclusions [1, 10, 12, 17, 18, 23, 24, 26].

The aim of the present study was to establish whether the biochemical parameters had a generally valid significance for judgment of the drug elimination ability and of the induced state.

Patients and methods

Thirty-five untreated male and female patients aged from 20 to 60 years, with liver disease of heterogeneous aetiology and various severity, were studied. Liver biopsy was performed in all of the cases. Of the 35 patients 8 had Gilbert's syndrome; 6 alcoholic fatty liver; 6 chronic persistent hepatitis; 6 chronic active hepatitis; and 9 had a decompensated alcoholic cirrhosis.

The methods for studying drug metabolizing capacity and enzyme induction used in this study have been described earlier [12]. Briefly they were as follows:

1. D-glucaric acid excretion in 24 h urine, using the method of Marsh [19]. Its result in liver disease may indicate hepatocellular damage or induction [7, 13, 14, 15, 16].

2. Menthol loading by oral administration of 2 g menthol, according to Szabó and Ébrey [30], using the analytical method of Bitter and Muir [4]. Urinary menthol glucuronide excretion gives information on glucuronic acid conjugation and it is suited for the estimation of induction [11, 30].

3. Antipyrine kinetic study by oral administration 20 mg/kg of the test drug. The methods of Brodie et al. [6] and Soberman et al. [28] were used to measure the biological half-life ($T_{1/2}$) as well as the metabolic clearance (MCR) of antipyrine [1, 2]. The method is suitable for assessing the hydroxylating function of the mixed function microsomal enzyme system [3, 22] and also for the measurement of induction [8].

4. Sulphadimidine kinetic study by i.v. administration of 1 g sulphadimidine, according to Talseth and Landmark [31], using the analytical method of Bratton and Marshall [32]. For the measurement of acetylating ability the biological half-life ($T_{1/2}$) of sulphadimidine was applied [23].

The correlations and relationships examined were as follows:

I. The correlation between serum albumin level (indicator of synthetizing ability of the liver) and microsomal functions (excretion of D-glucaric acid, menthol glucuronide, antipyrine $T_{1/2}$) was investigated.

II. Prothrombin and fibrinogen values were compared with the hydroxylating ability, reflected in the value of antipyrine MCR (metabolic clearance rate).

III. The value of SGOT an index of parenchymal lesion, was compared with the hydroxylating ability (antipyrine MCR).

IV. The serum bilirubin level was correlated to the acetylating ability (sulphadimidine $T_{1/2}$).

V. Gamma-GT activity was correlated to the endogenous and exogenous indicators of induction, the excretion of D-glucaric acid, and of menthol glucuronide. Their interrelation was also examined [8, 11, 20].

For evaluation of the results correlation analysis, variance analysis and the two-sample *t* test were used. The diagrams represent the diagnostic distribution of patients, and the correlation or the relationship between their given parameters.

Results

I. The only significant correlation of the serum albumin level was that with antipyrine kinetics ($T_1/_2$) (Fig. 1a, b, c).

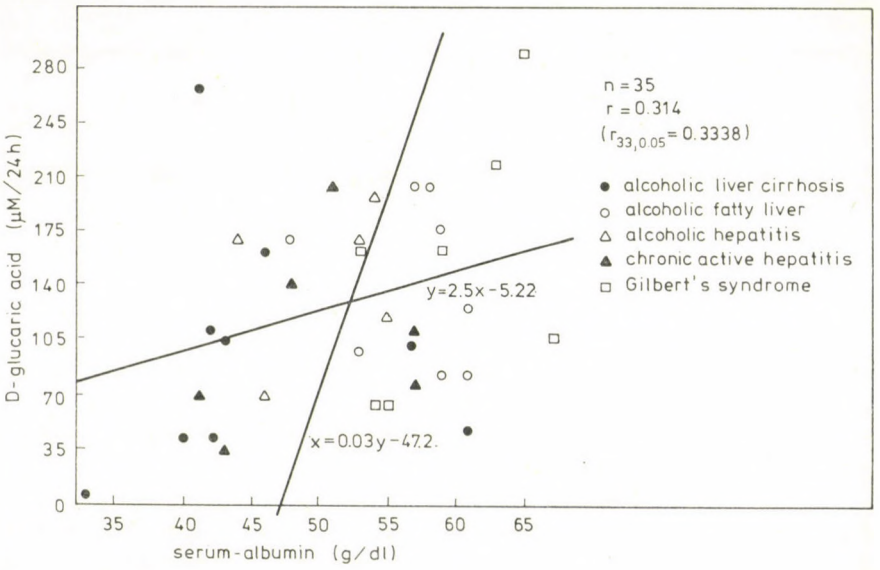

Fig. 1a.

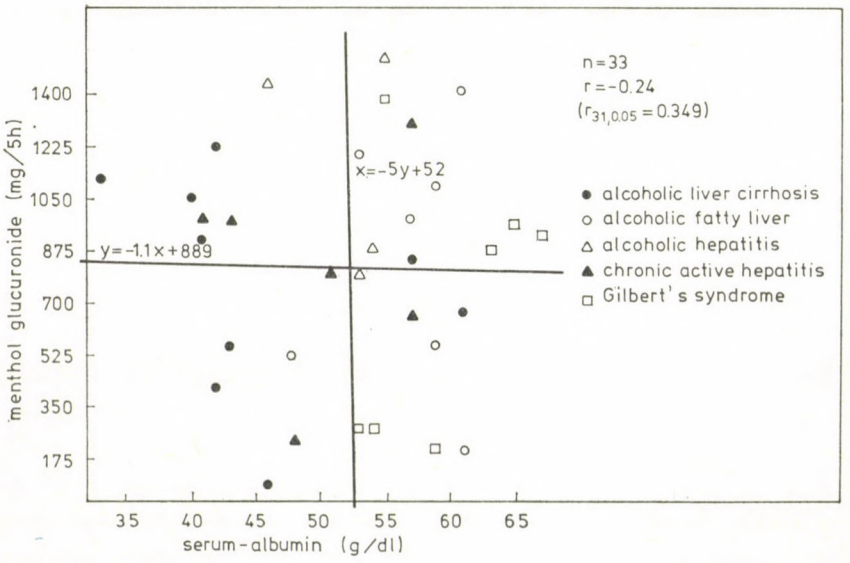

Fig. 1b.

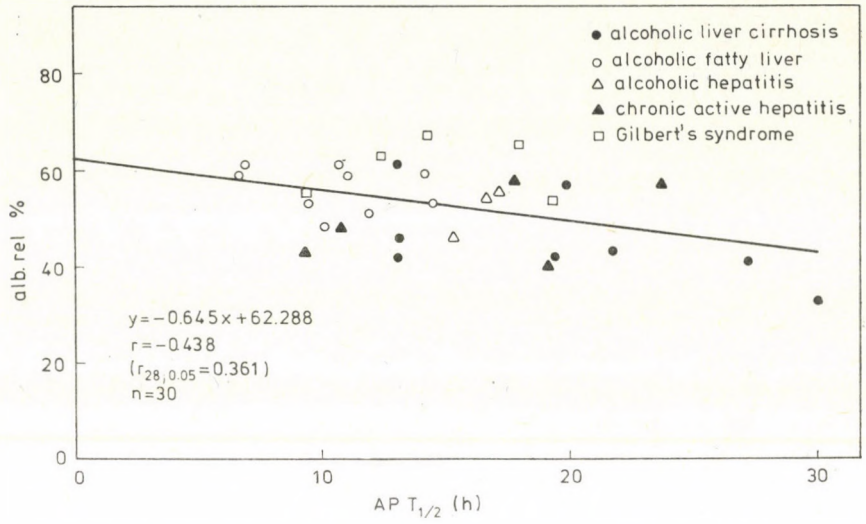

Fig. 1. Correlation between serum albumin and the microsomal functions activity, *a* D-glucaric acid excretion. *b* Glucuronide binding capacity. *c* Hydroxylating function. Significant correlation was found between serum albumin level and the hydroxylating function (antipyrine $T_{1/2}$)

II. The statistical differences between the mean antipyrine values for the normal and low prothrombin activities indicated that low prothrombin values indicate a major impairment of the hydroxylating ability (Fig. 2). The fibrinogen level may change in two directions; it may be elevated or lowered. The relationship of low fibrinogen levels with the hydroxylating capacity was the same as that of a low prothrombin activity (Fig. 3).

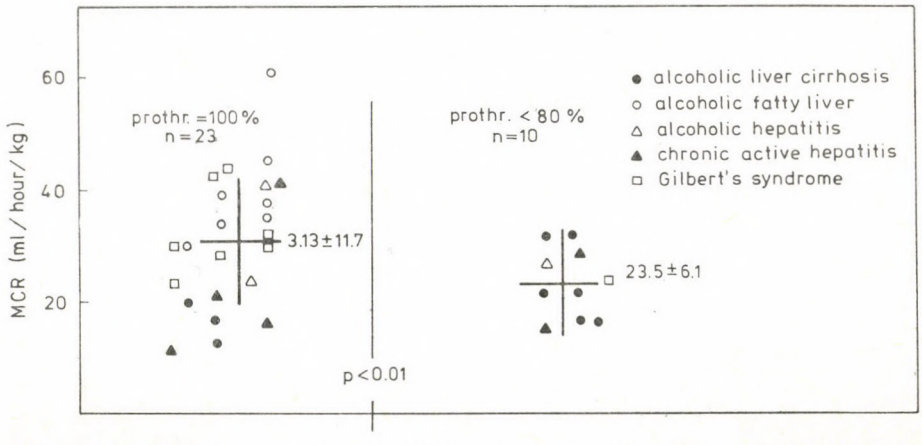

Fig. 2. Relationship between antipyrine MCR values and prothrombin activity: MCR values belonging to normal and pathological prothrombin values. The difference of hydroxylating ability between two groups is significant

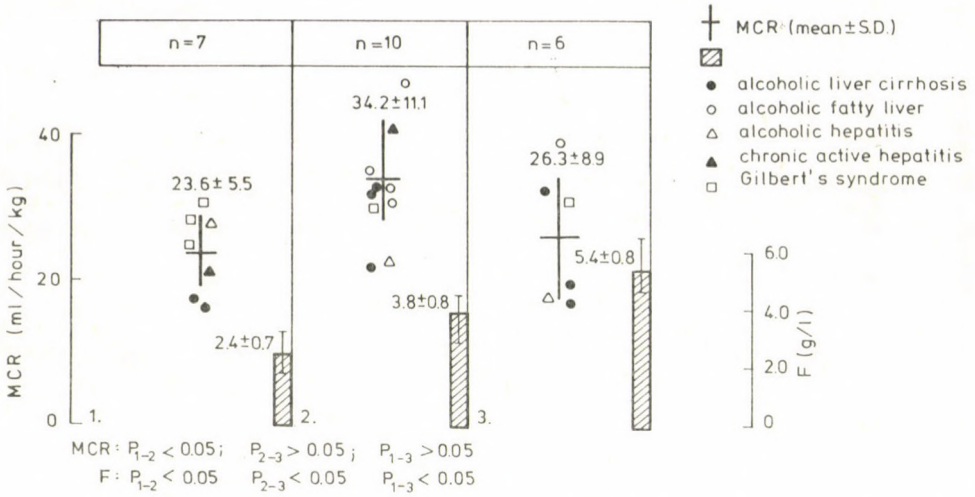

Fig. 3. Relationship between antipyrine MCR values and fibrinogen (F) level: MCR values belonging to lowered, nearly normal and elevated fibrinogen levels. Significant diminution of hydroxylating ability was found in the group with low fibrinogen level

III. SGOT is an important liver function test, but in connection with the present aim it was uninformative itself. Its biological significance depends on the severity and activity of the disease, and the same is the case with the hydroxylating ability (Fig. 4).

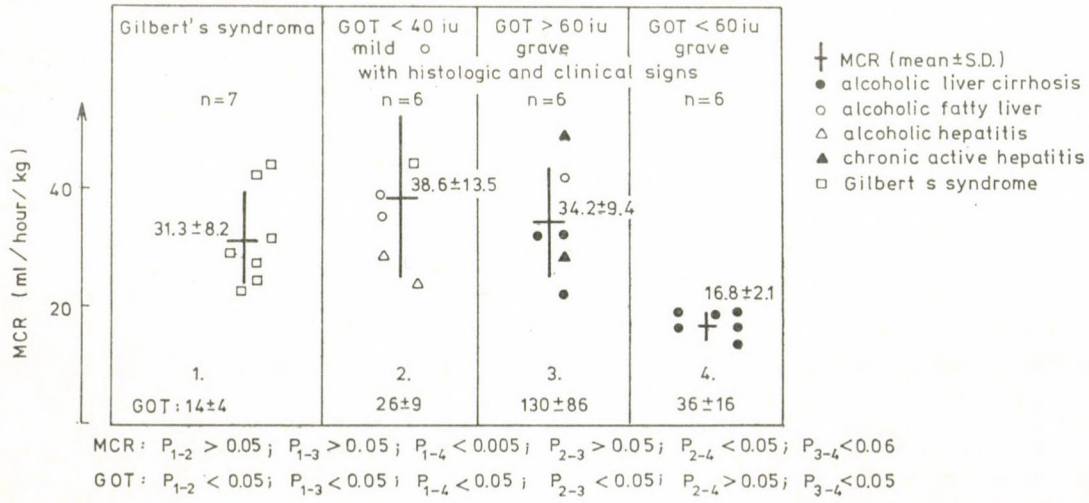

Fig. 4. Relationship between antipyrine MCR values and SGOT: SGOT values belonging to groups of damages with various biochemical activity. The differences of hydroxylating ability and SGOT values were parallel in comparison of groups only with extreme clinical and biological differences (1 and 4, 3 and 4)

IV. The correlation of the serum bilirubin level with the sulphadimidine $T_{1/2}$ was significant statistically, despite the small number of cases (Fig. 5).

V. There were no demonstrable connections between the three induction parameters in the group of heterogenous liver disease (Fig. 6a, b, c). This was, however, true only for a large group since the parameter may show a significant correlation in a smaller homogeneous group. Gamma-GT activity and D-glucaric acid excretion showed a close correlation in patients with alcoholic fatty liver (Fig. 7).

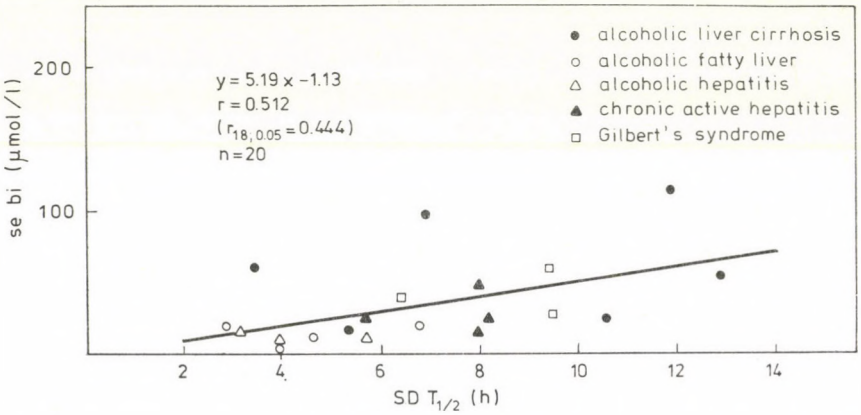

Fig. 5. Correlation between sulphadimidine kinetical parameter ($T_{1/2}$) and serum bilirubin level in patients with various chronic liver diseases. Significant correlation was found between serum bilirubin level and sulphadimidine elimination ($T_{1/2}$)

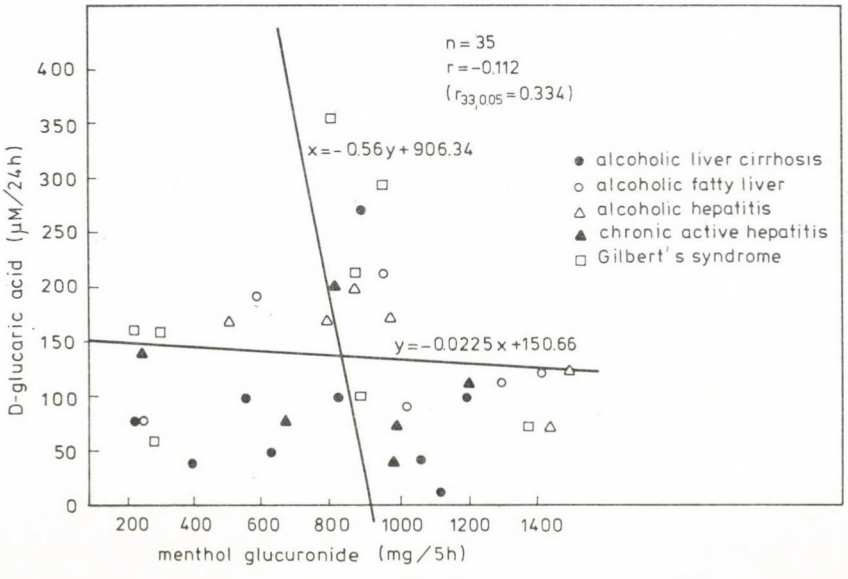

Fig. 6a.

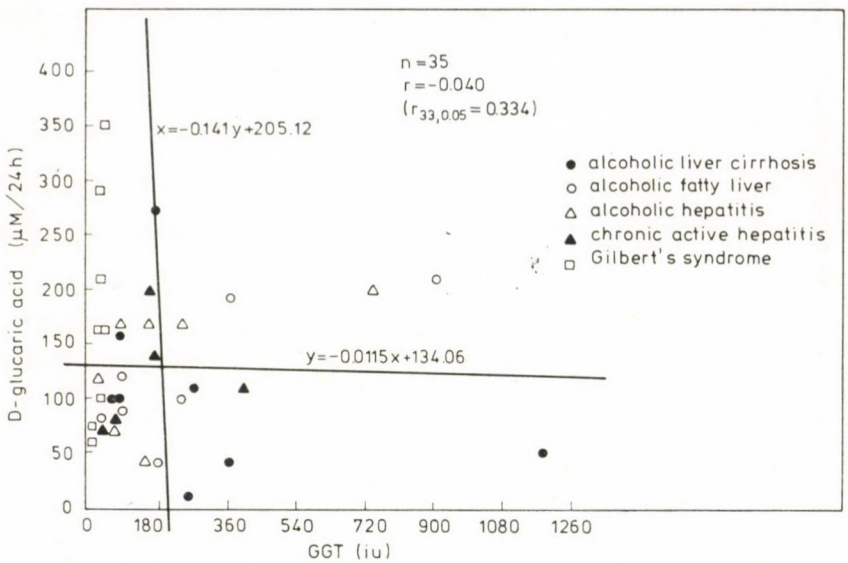

Fig. 6b.

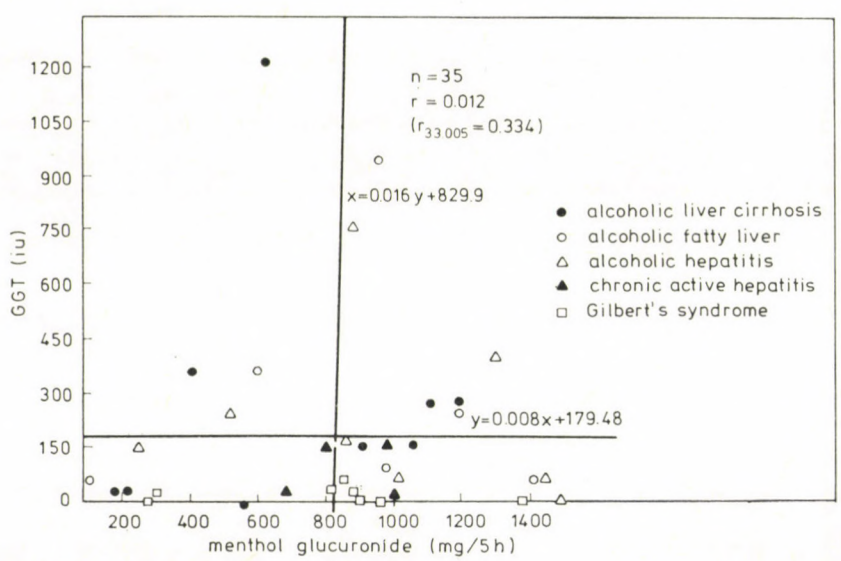

Fig. 6c.

Fig. 6. Correlation between enzyme induction parameters in groups of patients with various chronic liver disease: *a* D-glucaric acid excretion and glucuronide binding capacity. *b.* D-glucaric acid excretion and serum gamma-GT activity. *c.* Serum gamma-GT activity and glucuronide binding capacity. Correlation was not found between the three parameters of enzyme induction

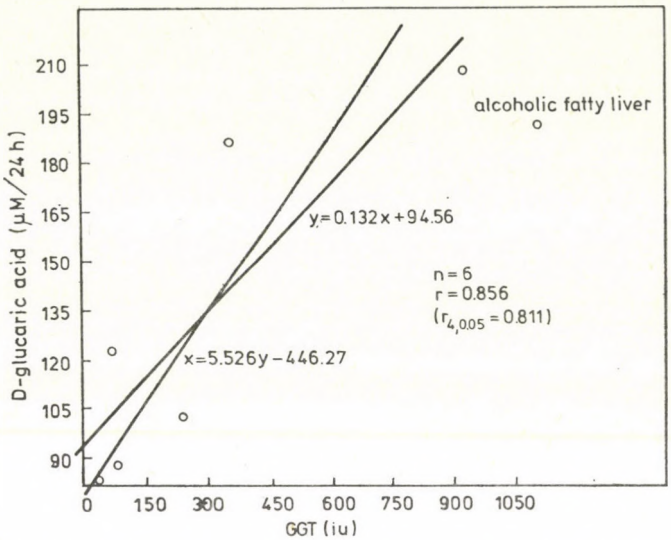

Fig. 7. Correlation between D-glucaric acid excretion and serum gamma-GT activity in a group of patients with alcoholic fatty liver. Positive correlation was found between these two parameters

Discussion

Numerous studies have been undertaken to simplify the assessment of the drug metabolizing ability in liver diseases [3, 5, 29]. Our correlation studies have also been concerned with this question. It has been attempted to use the de Ritis quotient for this purpose in viral hepatitis [7]. The variations of the elevated serum bilitubin level after treatment were widely used in Gilbert's syndrome for correlation studies for induction indicators [18]. A correlation has been demonstrated between D-glucaric acid excretion and the k_2 elimination constant of aminopyrine kinetics in normal individuals [15], and also between antipyrine kinetics (MCR) and the serum albumin level, between galactose elimination capacity and prothrombin activity and between antipyrine kinetics and urinary 6-beta-hydroxycortisol excretion [3,14]. The results of such studies are greatly influenced by the characteristics of the group, and by the previous treatment [10, 16, 22]. It is a special problem in liver diseases, in contrast to normal subjects, that the size of the liver is not in proportion to body size, neither correlates with the galactose elimination, nor with bromsulphophthalein transport. The reliability of the kinetic parameters may be improved by estimation of liver size and correcting the values according to the result [5].

Serum albumin provides a liver function parameter which shows progressively changing values in one direction only even in heterogenous groups.

(In the present material the serum albumin levels were given in per cents of the electrophoretic value because in cirrhosis the absolute values were influenced by water retention.) On the basis of its changes, the serum albumin level seems to provide the ideal basis for assessment of the drug eliminating ability, but our results did not support this. They only indicated that an impairment of the hydroxylating function, as reflected in the antipyrine $T_{1/2}$, is closely related to the decrease of the albumin level.

The relationships between abnormal prothrombin and fibrinogen values and drug elimination are known from earlier studies [3, 27]. It is, however, questionable whether the sensitivity of prothrombin activity approaches that of the albumin level. The bidirectional changes of the fibrinogen level cause difficulties in such correlation studies. SGOT values as indicators of parenchymal damage are of importance only if their clinical background is known. It is not irrelevant whether a slightly elevated GOT is found in liver disease of minor severity or in one of major severity. The sulphadimidine parameters (biological half-life, proportion of the acetylated fraction in serum and urine) show a behavior similar to that of INH, i.e. a bimodal distribution in the normal population. The mean biological half-life of the slow and rapid acetylators vary within certain limits, depending on the population under study and on the experimental circumstances [23, 33].

We have no sufficient explanation for the relationship between sulphadimidine $T_{1/2}$ and the serum bilirubin level found in the present study, because the protein binding has not been examined. It is, however, known that the elimination of compounds in an acetylated form is impaired in jaundice, thus 70% of the patients with Gilbert's disease were found to be slow acetylators [18, 26]. Levi et al., on the basis of kinetic studies of INH, which is also eliminated in acetylated form, found the prolongation of half-life in hepatic disease proportional to the increase in the serum bilirubin level, and the (INH-half-lives showed no bimodal distribution [17]. Having no information on protein binding the cause of the distribution is uncertain.

The significance of biochemical parameters in drug elimination is indisputable. However, in view of the multiplicity of interrelations, a given liver function test hardly seems to have such an informative value in itself, as it was observed between the serum bilirubin level and sulphadimidine kinetics. It seems more likely that the clinical pattern and the coexistence of biochemical tests indicating a well-defined liver damage may be distinctive from this point of view. The drug eliminating capacity may serve in this sense an additional liver function test. This interpretation of the relationships may constitute the basis of a discriminance analysis. Thus, it is felt that systematic prospective studies of well-defined groups of liver diseases have preserved their value in the investigation of relationships between biochemical tests and drug metabolizing ability. It seems that retrospective discriminance analysis will become more

informative with the use of more clinical tests and parameters of drug eliminating ability. A retrospective grouping of patients by the use of representative parameters may constitute the basis for the elaboration of rational dosage regime.

Acknowledgements

Our thanks are due to Miss Zsuzsanna Tatai, electrical engineer at the Central Laboratory of the University Medical School Pécs, for statistical analysis, and to the staff of the Central Clinical Chemical Laboratory for liver function tests.

REFERENCES

1. Ackermann, E.: Pharmakokinetik bei Leberzirrhose. Z. ges. inn. Med. **32,** 29–33 (1977).
2. Andreasen, P. B., Vessel, E. S.: Comparison of plasma levels of antipyrine, tolbutamide and warfarin after oral and intravenous administration. Clin. Pharmacol. Ther. **16,** 1059–1065 (1974).
3. Andreasen, P. B., Ranek, L., Statland, E. B., Tygstrup, N.: Clearence of antipyrine dependence of quantitative liver function. Europ. J. clin. Invest. **4,** 129–134 (1974).
4. Bitter, T., Muir, H. M.: A modified uronic acid carbazole reaction. Analyt. Biochem. **4,** 330–334 (1962).
5. Bircher, J., Blankart, R., Halpern, A., Hacki, W., Laissue, J., Preisig, R.: Criteria for assessment of functional impairment in patients with cirrhosis of the liver. Europ. J. clin. Invest. **3,** 72–75 (1973).
6. Brodie, B. B., Axelrod, J., Soberman, R., Levy, B. B.: The estimation of antipyrine in biological materials. J. biol. Chem. **179,** 25–29 (1949).
7. Carrella, M., D'Arienzo, A., Manzillo, G., DeRitis, F.: An evaluation of urinary D-glucaric acid excretion during acute hepatitis in man. Amer. J. digest. Dis. **23,** 18–21 (1978).
8. Davis, M., Simmons, C. J., Dordoni, B., Williams, R.: Urinary D-glucaric acid excretion and plasma antipyrine kinetics during enzyme induction. Brit. J. clin. Pharmacol. **1,** 253–257 (1974).
9. Eisenburg, J.: Diagnostische Interpretation von Serumenzym-Veränderungen bei Lebererkrankungen. Fortschr. Med. **86,** 849–852 (1968).
10. Hepner, G. W., Vesell, E. S., Lipton, A., Harvey, H. A., Wilkinson, G. R., Shenker, S.: Disposition of aminopyrine, antipyrine, diazepam and indocyanine green in patients with liver disease or on anticonvulsant drug therapy: diazepam breath test and correlations in drug elimination. J. Lab. clin. Med. **90,** 440–454 (1977).
11. Horváth, T., Gógl, Á., Simon, K.: Enzyme induction in man: a study of the inducible systems of drug elimination. Acta med. Acad. Sci. hung. **36,** 119–126 (1979).
12. Horváth, T., Pár, A., Past, T., Tapsonyi, Zs., Ruzsa, Cs., Kádas, I., Jávor, T.: Drug metabolism in patients with alcoholic liver disease as studied by test drugs and estimation of an endogenous metabolite. Acta med. Acad. Sci. hung. **39,** 169–177 (1982).
13. Hunter, J., Carrella, M., Maxwell, J. D., Stewart, D. A.: Urinary D-glucaric acid excretion as a test for hepatic enzyme induction in man. Lancet **1,** 572–575 (1971).
14. Latham, A. N., Turner, P., Franklin, C., Maclay, W.: Phenobarbitone induced urinary excretion of D-glucaric acid and 6-beta-hydroxycortisol in man. Canad. J. Physiol. Pharmacol. **54,** 778—782 (1976).
15. Lecamwasam, D. S.: Hepatic enzyme induction and its relationship to urinary D-glucaric acid excretion in man. Brit. J. clin. Pharmacol. **2,** 546–548 (1975).
16. Lecamwasam, D. S., Franklin, C., Turner, P.: Effect of phenobarbitone on hepatic drug-metabolising enzymes and urinary D-glucaric acid excretion in man. Brit. J. clin. Pharmacol. **2,** 257–262 (1975).
17. Levi, A. J., Sherlock, Sh., Walker, D.: Phenylbutazone and isoniazid metabolism in patients with liver disease in relation to previous drug therapy. Lancet **1,** 1275–1279 (1968).
18. Macklon, A. F., Savage, R. L., Rawlins, M. D.: Gilbert's syndrome and drug metabolism. Clin. Pharmacokin. **4,** 223–232 (1979).
19. Marsh, C. A.: Metabolism of D-glucuronolactone in mammalian systems. Identification of D-glucaric acid as a normal constituent of urine. Biochem. J. **86,** 77–86 (1963).

20. Martin, P. J., Martin, J. V., Goldberg, D. M.: Gamma-glutamyl transpeptidase, triglicerides and enzyme induction. Brit. med. J. **5,** 17–18 (1975).
21. Nilius, R., Neef, L., Otto, L., Zipprich, B., Busse, H. J., Peters, J. E., Speck, H. J.: Multivariate Untersuchungen zur Diskriminierungsfähigkeit von klinisch-chemischen Tests in der hepatologischen Diagnostik. Hepatologie **53,** 343–356 (1980).
22. Ohnhaus, E. E., Park, B. K.: Measurement of urinary 6-beta-hydroxycortisol excretion as in vivo parameter in the clinical assessment of the microsomal enzyme-inducing capacity of antipyrine, phenobarbitone and rifampicin. Europ. J. clin. Pharmacol. **15,** 139–145 (1979).
23. Van Outdtshorn, M. C. B., Potgieter, F. G.: Determination of pharmacokinetic parameters of rapid and slow acetylators of sulphadimidine. J. Pharm. Pharmacother. **24,** 357–360 (1972).
24. Paumgartner, G.: Der Einfluss von Lebererkrankungen auf Bioverfügbarkeit und Clearance von Medikamenten. Internist **21,** 718—723 (1980).
25. Pár, A., Mestyán, I., Kádas, I., Balázs, M., Gógl, Á.: Serum gamma-glutamyl transpeptidase: its clinical significance. Acta med. Acad. Sci. hung. **33,** 309–319 (1976).
26. Platzer, R., Küpfer, A., Bircher, J., Preisig, R.: Polymorphic acetylation and aminopyrin demethylation in Gilbert's syndrome. Europ. J. clin. Invest. **8,** 219–223 (1978).
27. Roberts, R. K., Branch, R. A., Desmond, P., Shenker, S.: The influence of liver diseases on drug disposition. Clin. Gastroent. **8,** 105–121 (1979).
28. Soberman, R., Brodie, B. B., Levy, B. B., Axelrod, J., Hollander, J., Steele, J. M.: The use of antipyrine in the measurement of total body water in man. J. biol. Chem. **179,** 31–42 (1949).
29. Sultatos, I. G., Dvorchik, B. H., Vesell, E. S., Shand, D. G., Branch, R. A.: Further investigations on relationship between antipyrine half-life, clearance, and volume of distribution: an appraisal of alternative kinetic parameters used to assess the elimination of antipyrine. Clin. Pharmacokin. **5,** 263–273 (1980).
30. Szabó, L., Ébrey, P.: Studies on the inheritance of Crigler-Najjar's syndrome by the menthol test. Acta paediat. Acad. Sci. hung. **4,** 153–158 (1963).
31. Talseth, T., Landmark, K. H.: Polymorphic acetylation of sulphadimidine in normal and uremic man. Europ. J. clin. Pharmacol. **11,** 33–36 (1977).
32. Varley, H.: Practical Clinical Biochemistry, 3rd ed., Heinemann, London 1962, pp. 501–507.
33. Vree, T. B., O'Reilly, W. J., Hekster, Y. A., Damsma, J. E., Van der Kleijn, E.: Determination of the acetylator phenotype and pharmacokinetics of some sulphonamides in man .Clin. Pharmacokin. **5,** 274–294 (1980).

Acta Medica Hungarica, Volume 40 (4), pp. 215—220 (1983)

Gastroenterology

GASTRIC ACID AND SERUM GASTRIN RESPONSE TO SHAM FEEDING, AND THE EFFECT OF CIMETIDINE ON THE RESPONSE TO SHAM FEEDING IN DUODENAL ULCER PATIENTS

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(Received November 29, 1982)

Sham feeding resulted in a significant increase of gastric acid secretion in 12 male patients with duodenal ulcer. No significant change in serum gastrin concentration was produced by sham feeding. Reproducibility of gastric acid response to sham feeding was very good ($r = 0.74$). The mean peak 30 min acid output amounted to 9.5 ± 1.0 mmol/30 min following sham feeding. That was 46.5% of the 30 min peak acid output elicited by pentagastrin infusion administered in a dose of 1.5 μg/kg/h. Cimetidine in a dose of 2 mg/kg/h almost completely reduced (by 85%) the gastric acid secretion induced by sham feeding. Cimetidine did not cause any change in serum gastrin concentration during and after sham feeding.

Keywords: duodenal ulcer, sham feeding, cimetidine, gastric acid, serum gastrin

Introduction

In duodenal ulcer patients the gastric acid response to sham feeding, the serum gastrin concentration during and after sham feeding, and the effect of cimetidine on sham feeding were investigated.

Materials and methods

Twelve male patients with duodenal ulcer participated in the study. They ranged in age from 22 to 54 (mean 36.4) years and their mean weight was 65.6 kg.

Duodenal ulcer was diagnosed on the basis of an ulcer crater in barium studies and by endoscopy. Antisecretory drugs were discontinued at least three days before the experiments. After and overnight fast a nasogastric tube was placed in the antrum. In the first hour, basal acid secretion was measured, then the subjects were fed a test meal consisting of 200 g fried pork, 250 g french-fried potatoes and 200 ml water. The test meal was seasoned with salt and pepper. A "modified sham feeding" test [14, 16] by "chew and spit" technique was used. The patients tasted the meal, and chewed it for 30 min. They were instructed not to swallow the food and had to rinse their mouth with water after every morsel. During all tests gastric aspirates were checked for swallowed food particles and none was found.

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In studies with pentagastrin, an intravenous infusion of pentagastrin (Peptavlon, I.C.I.) was given in a constant dose of 1.5 μg/kg/h for 60 minutes. This dose of pentagastrin usually elicits the maximal secretory rate in duodenal ulcer patients [5].

The variability in acid output provoked by sham feeding was examined after a 4-day interval. The regression line was calculated by the method of least squares. Linear regression equation, correlation coefficient and significance were given.

In studies with sham feeding and cimetidine, an intravenous infusion of cimetidine (Tagamet) was administered with a dose of 2 mg/kg/h for one hour. The infusion was started at the beginning of sham feeding.

Gastric secretion was collected by an electric suction pump in 15 min periods. The volume of gastric secretion was measured and hydrogen ion concentration was determined by a glass electrode. Potentiometric titration to pH 7.0 with 0.1 N NaOH was used. Acid output in 15 min periods was calculated in millimoles (mmol per 15 min). The results were expressed as mean and ± S.E.M.

The 30 min peak acid output (PAO) in response to sham feeding and pentagastrin infusion was expressed in the sum of the two highest consecutive 15 min gastric acid outputs following the start of the tests.

In sham feeding and cimetidine experiments the percentage of inhibition was calculated by expressing the 30 min PAO during sham feeding alone and in the same periods the mean 30 min acid output during sham feeding and cimetidine. The significance of differences in the 15 min acid outputs was tested by *t* test.

Venous blood for determining serum gastrin concentrations was collected through an indwelling catheter, which was kept open by slow saline infusion. Blood samples were obtained at 15 min intervals before, during and after sham feeding. Blood was stored at —20 °C until assayed. All samples were tested by CIS CEA SORIN kit in duplicate in the same assay. The basal serum gastrin concentration was defined as the mean of the two serum gastrin levels measured before sham feeding.

Results

As shown in Fig. 1, acid secretion was stable during the 60 min. basal period. During sham feeding acid secretion increased and reached the peak during the second 15 min. period of sham feeding. During the next 30 min, acid secretion decreased. Acid rises in response to sham feeding were significant above basal levels.

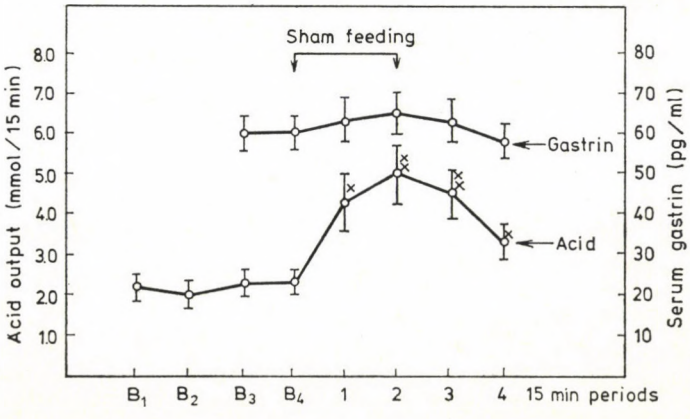

Fig. 1. Effect of sham feeding on serum gastrin level and gastric acid secretion in 12 duodenal ulcer patients. Asterisks indicate significant differences.) * $P < 0.05$, ** $P < 0.01$

Sham feeding caused only a small rise in serum gastrin concentration. The peak gastrin increment was reached by the end of sham feeding, i.e. at 30 min but it was not significant as compared to the basal gastrin level.

Reproducibility of the gastric acid response to sham feeding in the first hour in the 12 duodenal ulcer patients is shown in Fig 2. A highly significant ($p < 0.001$) correlation ($r = 0.74$) was observed between results of the first and second tests.

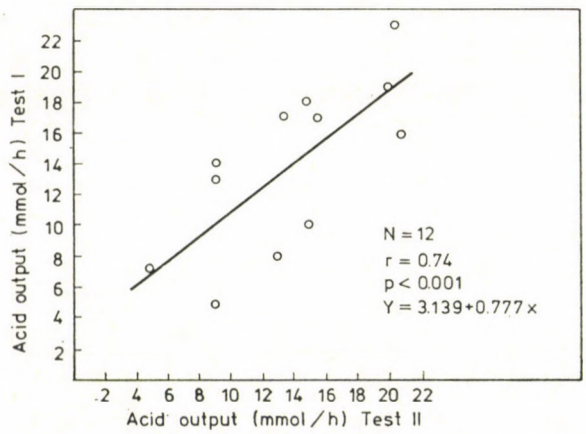

Fig. 2. Reproducibility of gastric acid secretion evoked by sham feeding. Each point represents results of two tests in one subject

Figure 3 illustrates the 30 minute mean gastric acid secretory responses (PAO) to sham feeding and pentagastrin infusion in the 12 duodenal ulcer patients. PAO amounted to 9.5 ± 1.0 mmol/30 min following sham feeding, corresponding to 46.5% of the mean 30 min peak acid output to pentagastrin (20.4 ± 1.55 mmol/30 min).

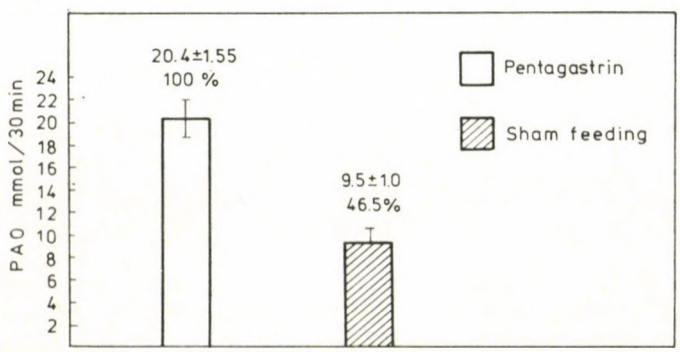

Fig. 3. Peak acid outputs produced by pentagastrin infusion given in a dose of 1.5 μg/kg/h, and by sham feeding in the 12 duodenal ulcer patients

Figure 4 shows that cimetidine infusion almost completely abolished the acid response to sham feeding. Cimetidine produced no change in the serum gastrin level observed during and after sham feeding. The acid inhibition was 85%.

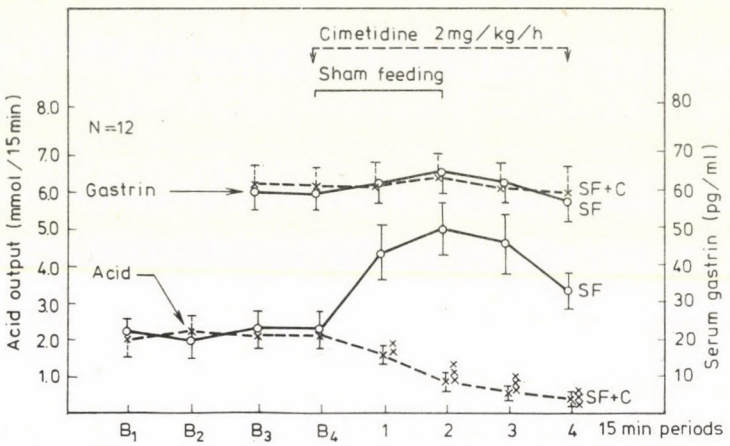

Fig. 4. Effect of cimetidine on gastric acid secretion stimulated by sham feeding and on the serum gastrin level during and after sham feeding. Solid lines represent effects of sham feeding as controls in the 12 patients with duodenal ulcer. Asterisks indicate significant differences. ** $P < 0.01$, *** $P < 0.001$

Discussion

Sham feeding is probably the most physiological way to induce vagal gastric acid secretion by activating efferent vagal pathways to the stomach. In the present study sham feeding resulted in a marked and significant increase of gastric acid secretion in duodenal ulcer patients. Our results are in agreement with the observations of other authors who found a similar significant acid response to sham feeding in healthy humans [3, 7, 10, 11, 13] and in duodenal ulcer patients [3, 6, 7, 8, 10, 12, 14, 16]. We found that sham feeding did not cause a significant increase in serum gastrin concentration in duodenal ulcer patients when the stomach contents were aspirated. Our results confirm the reports of several authors [7, 8, 9, 11, 15, 16] who found no significant change in serum gastrin level following sham feeding but disagree with some workers [1, 2, 4, 6, 10] who were able to demonstrate a significant increase of the serum gastrin concentration during sham feeding. The reason for the discrepancy is not known. It was suggested that the standard aspiration technique probably allows some acid to bathe the antral mucosa which may produce a feedback inhibition of gastrin release [13]. Namely, in previous studies when the intragastric pH was kept at 5.0 by in vivo intragastric titration during sham feeding

in man, there was a significant increase in the serum gastrin level [1, 2]. On the other hand, when the intragastric pH was maintained at 2.5, there was no gastrin response to sham feeding [2]. In contrast with these studies, Stenquist et al. [16] and Konturek et al. [8] were unable to confirm this suggestion. These authors maintained the intragastric pH between 6.1 and 8.1 [16] or at 5.5 [8] and did not find any significant increase in the serum gastrin level during sham feeding.

Our finding that the serum gastrin response to sham feeding was not significant indicates that the vagal release of gastrin is of minor importance for gastric acid secretion stimulated by sham feeding in duodenal ulcer patients. This observation may be interpreted so that vagal excitation induces gastric acid secretion almost entirely by direct cholinergic activation of the parietal cells.

Reproducibility of the gastric acid response to sham feeding revealed a close correlation ($r = 0.74$) between the different variables. No significant difference was detected between the acid responses obtained in duplicate tests with sham feeding in 12 duodenal ulcer patients.

PAO during sham feeding was 46.5% as expressed as a percentage of PAO to pentagastrin infusion given in a dose of 1.5 μg/kg/hr.

In 12 patients with duodenal ulcer the acid response to sham feeding was almost totally abolished by administering cimetidine in a dose of 2.0 mg/kg/h. The inhibition of gastric acid secretion amounted to 85%. Cimetidine caused no change in the serum gastrin level during sham feeding.

REFERENCES

1. Feldman, M., Richardson, C. T., Taylor, I. L., Walsh, J. H.: Effect of atropine on vagal release of gastrin and pancreatic polypeptide. J. clin. Invest. **63,** 294—298 (1979).
2. Feldman, M., Walsh, J. H.: Acid inhibition of sham feeding-stimulated gastrin release and gastric acid secretion: effect of atropine. Gastroenterology **78,** 772—776 (1980).
3. Feldman, M., Richardson, C. T., Fordtran, J. S.: Effect of sham feeding on gastric acid secretion in healthy subjects and duodenal ulcer patients: evidence for increased basal vagal tone in some ulcer patients. Gastroenterology **79,** 796—800 (1980).
4. Feldman, M., Richardson, C. T.: Sham feeding releases gastrin in man. Gastroenterology **78,** 1164 (1980).
5. Kisfalvi, I.: Pentagastrin-infusióval végzett vizsgálatok fekélybetegekben. Magy. Belorv. Arch. **29,** 16—20 (1976).
6. Knutson, V., Olbe, L., Ganguli, P. C.: Gastric acid and plasma gastrin responses to sham feeding in duodenal ulcer patients before and after research of antrum and duodenal bulb. Scand. J. Gastroent. **9,** 351—356 (1974).
7. Konturek, S. J., Kwiecien, N., Obtulowicz W., Miklós, E., Sito, E., Oleksy, J., Popiela, T.: Cephalic phase of gastric secretion in healthy subjects and duodenal ulcer patients: role of vagal innervation. Gut **20,** 875—881 (1979).
8. Konturek, S. J., Obtulowicz, W., Kwiecien, N., Sito. E., Miklós, E., Oleksy, J.: Comparison of ranitidine and chimetidine in the inhibition of histamine, sham feeding, and meal-induced gastric secretion in duodenal ulcer patients. Gut **21,** 181—186 (1980).
9. Konturek, S., J. Obtulowicz, W.. Kwiecien N., Dobrzanska, M,. Swierczek, J., Kopp, B., Olesky, J.: Effect of pirenzepine and atropine on gastric secretory and plasma hormonal responses to sham feeding in patients with DU. Scand. J. Gastroent. **15,** Suppl. 66, 63—69 (1980).

10. Mayer, G., Arnold, R., Feurle, G., Fuchs, K., Ketterer, H., Track, N. S., Creutzfeldt, W.: Influence of feeding and sham feeding on serum gastrin and gastric acid secretion in control subjects and DU patients. Scand. J. Gastroent. **9,** 703—710 (1974).
11. Mignon, M., Galmiche, J. P., Accary, J. P., Bonfils, S.: Serum gastrin, gastric acid and pepsin responses to sham feeding in man. Gastroenterology **66,** 856 (1974).
12. Olbe, L., Knutson, V.: Gastric acid response to sham feeding in the dog and the duodenal ulcer patient. Acta hepato-gastroent. (Stuttg.) **23,** 455—458 (1976).
13. Richardson, C. T., Walsh, J. H., Cooper, K. A., Feldman, M., Fordtran, J. S.: Studies on the role of cephalic-vagal stimulation in the acid secretory response to eating in normal human subjects. J. clin. Invest. **60,** 435—441 (1977).
14. Stenquist, B., Knutson, V., Olbe, L.: Gastric acid responses to adequate and modified sham feeding and to insulin hypoglycemia in duodenal ulcer patients. Scand. J. Gastroent. **13,** 357—362 (1978).
15. Stenquist, B., Rehfeld, J. F., Olbe, L.: Effect of proximal gastric vagotomy and anticholinergics on the acid and gastrin responses to sham feeding in duodenal ulcer patients. Gut **20,** 1020—1027 (1979).
16. Stenquist, B., Nilsson, B. S. G., Rehfeld, J. F., Olbe, L.: Plasma gastrin concentrations following sham feeding in duodenal ulcer patients. Scand. J. Gastroent. **14,** 305—311 (1979).

Acta Medica Hungarica, Volume 40 (4), pp. 221—227 (1983)

MICROBIOLOGICAL FINDINGS AND PROTEIN CONCENTRATION IN GASTRIC JUICE

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(Received May 17, 1982)

From 17 patients subjected to pentagastrin test, 136 samples of gastric juice (fasting sample, basal secretion, fractions after stimulation) were collected. The concentration of the protein components (IgG, IgA, IgM, C3 and albumin) in the fasting samples were in excess of those found in the basal secretion, but protein output (volume × concentration) was nearly identical in the two samples. The protein concentration of the fractions obtained in response to pentagastrin stimulation were too low to be measurable. According to correlation analysis, protein concentration in the gastric juice is primarily the function of the microbiological finding. The allergic effect of microorganisms in the gastric juice may give rise to an increase in its immunoglobulin and albumin concentrations

Keywords: pentagastrin test, gastric juice, microbiological findings, immunoglobulins, immune response

Introduction

The digestive system is a rich source of living (microbial) and dead (nutritive, microbial) antigens. The mucous membranes are in continuous contact with countless antigens and allergens. The gastrointestinal microflora is under the control of multiple factors. Nonspecific factors of regulatory influence include peristalsis, proteolytic enzymes, lysozyme, conjugated bile acids, bacterial antagonism or synergism and gastric acid [1]. The specific factors are provided by secretory immunoglobulins (IgA, IgM) attaining the mucosal immune system [13]. It is due to the complex effect of these factors that 33% of the gastric and 20% of the duodenal juice are sterile [4], though the reservoir function of the stomach somewhat inhibits the defence mechanism of the gastric mucosa. The highest microbial counts have been found in achlorhydric cases [12] and Varró regards E. coli contamination of the gastric juice as inseparable from achlorhydria [14].

In gastric achlorhydria or hypochlorhydria the incidence of duodenal colonization of pathogens is also high. The microbial populations of the hypochlorhydric or achlorhydric gastric juice find easy access to the duodenum in

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this way [7]; the regulatory effect of gastric acidity thus extends to the duodenal microflora as well [2].

The digestive activity of the proteolytic enzymes also belongs to the factors accounting for the absence of proteins and antigens from the normochlorhydric gastric juice [10]. This implies that the immunoglobulins attaining the surface of the gastric mucosa display their defence mechanism only in certain situations. Under physiological conditions proteolysis of antibodies may even take place. Some twenty years ago Heiskell et al. [3] raised the possibility that in gastric malignancy the mechanism accounting for the wide variety of proteins in the gastric juice must be a more complex one than a simple haemorrhage. Their findings have been confirmed by further studies [8, 9, 16].

Earlier [11] we have found striking differences between hypo- or achlorhydric and hyper- or normochlorhydric samples in this respect. The results of microbiological studies were closely related to the acidity of gastric juice [10, 11], the microbiological finding having been invariably positive in the cases of hypochlorhydria or achlorhydria, but only in a small proportion of those of hyperchlorhydria or normochlorhydria. Moreover, in all cases in which microorganisms had been found in the gastric juice, the immunoglobulin and albumin levels of all fractions were higher than in the case of negative bacteriological findings. The highest immunoglobulin concentrations were found in the presence of a mixed bacterial flora. The relationship between the increase in the immunoglobulin concentrations and microbiological findings was no confined to the gastric juice; it was also demonstrable in the duodenal fluid (bile fractions A, B, C) [11].

As it is known, the smallest gastric volumes are observed in the case of achlorhydria. It was therefore justified to examine the question how far the composition of proteins and microorganisms in the gastric juice is determined by their concentration. We have therefore subjected to a comparative study the fractions obtained during a pentagastrin test, to identify the factors accounting for the increase in protein concentration. Thus, the relation between the bacterial count, the protein concentration and the parameters of acidity (chemical reaction, HCl concentration, acid secretory responsive) has been examined.

Patients and methods

Seventeen patients, 13 with duodenal ulcer and 4 with gastric symptoms with no organic abnormality, were subjected to a pentagastrin test (Acignost®, Berlin-Chemie, GDR 6 μg/kg subcutaneously). Gastric juice was withdrawn first in the fasting state, followed by collection of fractions at 15 min intervals over 1 h prior to pentagastrin stimulation (BAO: basal acid output), then for another hour after pentagastrin stimulation (MAO: maximal acid output). A total of 136 samples was examined. Diagnosis was based on endoscopic or X-ray evidence in each case. No ulcerogenic drugs (salicylates, antirheumatic or antihypertensive agents, steroids) had been administered, and no abnormalities of the parenchymal, endocrine or blood-forming organs were demonstrable in any of the cases.

In each fraction the concentration of IgG, IgA, IgM, complement C3, albumin (g/l), volume, chemical reaction, HCl concentration (mmol/l) and acid secretory response (mmol/15 min or h) was determined, then the fasting gastric juice and the basal secretion were studied for microorganisms. In the calculations for chemical reaction first the absolute ion concentration was considered and reconverted to the pH value. For quantitative study of the immunoglobulins and the other protein components our method described earlier [10, 11] was used, its sensitivity having been 0.01 g/l.

Microbiological studies. The bacteria were counted and identified. For aerobic cultures 0.1 ml aliquots of gastric juice were measured onto the surface of blood-agar plates containing 5% bovine blood and of eosin-methylene blue agar plates, and spread evenly over the surface. After incubation at 37 °C for 24 h and at room temperature for another 24 h the colonies were counted and the bacteria were identified by microscopic and biochemical methods. The results were referred to 1 ml gastric juice. For anaerobic cultures, 0.1 ml aliquots of gastric juice were measured onto glucose-containing blood-agar Lab-Lemco medium and evenly spread over the surface. Anaerobic conditions were provided by the alkaline pyrogallol method. After incubation at 37 °C for 72 h the colonies were counted and the bacteria were identified by microscopic and biochemical evidence methods. The results were referred to 1 ml material.

Biostatic methods. Mean values ± S.D. were considered; for correlation studies Student's two sample *t* test was used.

Results

The samples taken after pentagastrin stimulation (MAO fractions) contained no immunoglobulins, complement C3 or albumin measurable by the methods used.

Albumin concentration in the fasting samples was found to exceed that of the basal secretions (Fig. 1, Table I). Protein output (volume × protein concentration) was nearly identical in the fasting sample and the basal secretions (Table II), the difference between them was not significant. The immunoglobulin/albumin-proportion in the fasting sample and the basal secretion was also practically identical (Table II).

Results have been assembled in Tables III and IV. There was a close correlation between the acid secretory response (mmol/h) and HCl concentra-

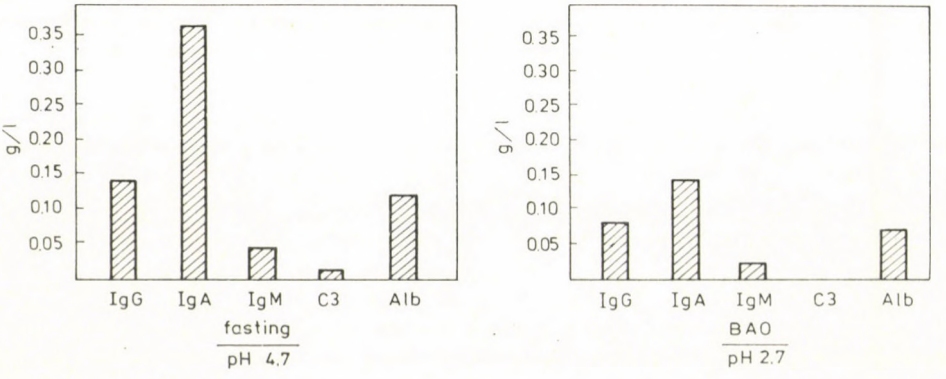

Fig. 1. Immunoglobulins, C3 and albumin in fasting sample (F) and basal secretion (BAO) in pentagastrin test. Immune sera: "Humán", Budapest; for C3 "Sevac", CSSR

Table I

Protein concentration (g/l) and bacterial counts in fasting sample (F) and basal secretion (BAO), mean ±S.D.

	F	BAO
IgG	0.14 ± 0.25	0.08 ± 0.21
IgA	0.36 ± 0.55	0.14 ± 0.34
IgM	0.04 ± 0.07	0.02 ± 0.05
C3	0.01 ± 0.01	0.01 ± 0.01
Albumin	0.12 ± 0.17	0.07 ± 0.16
Bacterial count	$1.5 \times 10^4 \pm 3.2 \times 10^4$	$1.5 \times 10^3 \pm 3.2 \times 10^3$

Table II

Protein output (volume × concentration) in fasting sample (F) and basal secretion (BAO), (g)

	F	BAO
IgG	0.0046	0.0059
IgA	0.0118	0.0104
IgM	0.0013	0.0015
C3	0.0002	0.0002
Albumin	0.0039	0.0044

Proportion of protein components: IgG/IgA $\approx$ 1 : 2; IgA/albumin $\approx$ 2.5 : 1; IgG/albumin $\approx$ 1 : 1

Table III

Correlation of data from fasting sample (F) and basal secretion (BAO) ($n = 17$; $P < 0.01$)

F IgM/BAO IgA	$r = 0.8894$
F IgM/BAO IgG	$r = 0.6510$
F IgM/BAO IgG	$r = 0.7888$
F IgM/BAO C3	$r = 0.7917$
F IgM/BAO albumin	$r = 0.7596$
BAO IgG/BAO IgM	$r = 0.7090$
BAO IgG/BAO C3	$r = 0.8351$
BAO IgG/BAO albumin	$r = 0.9828$
BAO IgA/BAO IgM	$r = 0.9423$
BAO IgM/BAO C3	$r = 0.9723$

Table IV

Correlation of the data from fasting sample (F) and basal secretion (BAO) ($n = 17$; $P < 0.01$)

F IgM/BAO bacterial count	$r = 0.8230$
BAO bacterial count/BAO IgG	$r = 0.8507$
BAO bacterial count/BAO IgA	$r = 0.8937$
BAO bacterial count/BAO IgM	$r = 0.9664$
F bacterial count/BAO C3	$r = 0.9939$
BAO bacterial count/BAO albumin	$r = 0.9278$
BAO pH/BAO bacterial count	$r = 0.7439$

tion (mmol/l); the r being nearly 1.0. As expected, the immunoglobulins in the samples correlated closely with each other, the complement C3 and the albumin (Table III). For instance, a close correlation between the proteins of vascular origin was reflected by the r of 0.9829 between IgG and BAO albumin or by the r of 0.9126 between complement C3 and albumin. A similarly close correlation was found in basal secretion between IgM (which is also formed locally in the mucosa and takes part in the complement binding reaction) and C3 ($r = 0.9723$). In the basal secretion the correlation between the immunoglobulins formed locally was also very close, IgA and IgM (g/l) giving an r value of 0.9423.

The bacterial counts were also found to correlate closely with the concentration of the individual immunoglobulins and of complement C3 (Table IV): $r = 0.9939$ between the fasting bacterial counts and complement C3 (g/l) concentration of BAO; $r = 0.9664$ between the bacterial counts and IgM (g/l) of BAO; $r = 0.8937$ between the bacterial counts and IgA (g/l) of BAO; $r = 0.9278$ between the bacterial counts and albumin (g/l) of BAO.

A close correlation was demonstrable also between the pH and the bacterial counts of BAO; $r = 0.7439$.

Discussion

As pointed out by Kétyi et al. [5, 6], a gross and persistent disturbance of the intestinal flora is in itself no pathogenic factor. In our view it is due to the specific protective effect of the mucous membranes which are not accustomed to an otherwise persistent microbial colonization or to the presence of pathogenic microorganisms, and which can cope with the massive, biologically active microbial populations without any gross damage. It is also due to

the live mucosal products that the achlorhydric gastric contents may remain sterile [14]. It has been confirmed by van der Waaij [15] that the majority of bacteria forming the intestinal flora in mice are coated in vivo with secretory immunoglobulins.

On the evidence of the present study, the bacterial counts correlate very closely with the concentrations of the individual immunoglobulins and of complement C3, the *r* being approximately 1.0. Our earlier studies [11] also revealed close correlations between the immunoglobulin concentrations of the fractions fo gastroduodenal juice and the presence of microorganisms. It may be assumed on these grounds that the immunoglobulin concentrations of the body fluids primarily result from immunogenic microbial factors. In the light of these findings, the presence of microorganisms in the body fluids, owing to their allergenic properties, is by no means indifferent to the body. Therapeutic measures in accordance with the bacteriological finding are, therefore, certainly justified.

The protein concentration of stimulated glandular secretions is known to be below the basal value [13]. A stimulation of major intensity may result in highly diluted secretions with so low protein concentrations the measurement of which requires quantitative methods of particular sensitivity. This may account the present observation that the protein components revealed the highest concentration in the fasting sample and in the basal secretion, but after pentagastrin stimulation they were not measurable by the methods used. As pointed out earlier, the differences between fasting sample and basal secretion in the concentrations of the protein components are eliminated by taking the protein output into consideration.

The reported results point to the significance of mucosal infections. A possible increase in the immunoglobulin concentration of the body fluids is primarily due to the allergic effect of the microorganisms identifiable on the mucous membranes. This presupposes a specific protective capacity of the mucous membrane which assumes a compensatory activity against the disturbances of acidity and proteolytic activity associated with chronic processes.

REFERENCES

1. Brandtzaeg, P.: Factors involved in host protection against infections. In: Mc Phee (ed.): Host Resistance to Commensal Bacteria. Churchill Livingstone, Edinburgh 1972.
2. Gray, J. D., Shiner, M.: Influence of gastric pH on gastric and jejunal flora. Gut **8,** 574–581 (1967).
3. Heiskell, C. L., Wada, T., Stempien, S. J., Fukuda, M., Kakagawa, S., Yachi, A., Dagradi, A., Carpenter, C. M.: Normal serum proteins in gastric juice. Gastroenterology **40,** 775–781 (1961).
4. Hruby, S., Maresová, P., Zezulková, M.: Zur Problematik der Verdauungssäfte in ihrer Eigenschaft als Antimikrobial-barriere. In: Knoke, M., Bernhardt, H. (eds): Gastrointestinale Mikroflora des Menschen. J. A. Barth, Leipzig, 1980, pp. 106–108.
5. Kétyi I., Barna K.: A humán bélflórára vonatkozó vizsgálatok. I. A normál bélflórával és állandó komponenseinek stabilitásával kapcsolatos megfigyelések. Magy. belorv. Arch. **4,** 51–62 (1965).

6. Kétyi I., Barna K.: A humán bélflórára vonatkozó vizsgálatok. II. Antibiotikum kezelés hatására létrejött változások a humán bélflórában. Magy. belorv. Arch. **5,** 67–80 (1965).
7. Knoke, M., Bernhardt, H.: Magensaftazidität und Hefepilzbesiedlung des Duodenum. Mykosen **20,** 371–379 (1977).
8. Rapp, W., Aronson, S. B., Burtin, P., Grabar, P.: Constituents of antigens of normal human gastric mucosa as characterized by immunoelectrophoresis in agar gel. J. Immunol. **92,** 579–595 (1964).
9. Simons, K., Gräsbeck, R.: Immunoelectrophoresis of human gastric juice. Clin. chim. Acta **8,** 4252433 (1963).
10. Somos, Zs., Loibl, A., Gróf, P.: The local immunity is a part of the gastrointestinal defense mechanism. In: Gy. Mózsik, O. Hänninen, T. Jávor (eds), Gastrointestinal Defence Mechanisms. Advanc. physiol. Sci. **29,** 511–539 (1980).
11. Somos, Zs., Loibl, S., Gróf, P.: Protein content of gastroduodenal juice: concentration and significance. Acta med. Acad. Sci. hung. **38,** 135–143 (1981).
12. Stockbrügger, R., Kastrup, W., Lundquist, G., Mobachen, H.: Development of gastric dysfunction in dermatitis herpetiformis. Acta dermatovenereol. (Stockh.) **58,** 343–348 (1978).
13. Tomasi, T. B. Jr.: The Immune System of Secretions. A. G. Osler (ed.) Prentice Hall, Englewood Cliffs 1976.
14. Varró V.: Achlorhydria. Akadémiai Kiadó, Budapest 1964.
15. van der Waaij, D.: The role of the microbial flora in the development, maintance and modulation of host immune function. In: Mózsik, Gy., Hänninen, O., Jávor, T. (eds): Gastrointestinal Defence Mechanisms. Advances in Physiol. Sciences. vol. 29. Akadémiai Kiadó, Budapest 1981 pp 541—551.
16. Wurster, K., Kuhlmann, W. D., Rapp, W.: Immunohistochemical studies of human gastric mucosa. Procedures for routine demonstration of gastric proteins by immunoenzyme technique. Virchows Arch. path. Anat. **378,** 213-228 (1978).

Acta Medica Hungarica, Volume 40 (4), pp. 229—237 (1983)

SECRETIN-PANCREOZYMIN TEST WITH SYNTHETIC SECRETIN AND CHOLECYSTOKININ OCTAPEPTIDE

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(Received December 22, 1982)

Pancreatic responses to submaximal doses of synthetic secretin (125 ng/kg) and cholecystokinin octapeptide (50 ng/kg) were investigated in controls and patients with pancreatic disease. Doses of stimulants were chosen from dose-response experiments to avoid supramaximal amounts which inhibited the pancreatic response.

Injection of secretin resulted in duodenal juice in less trypsin but more lipase activity than that elicited by cholecystokinin octapeptide. The increase of amylase was about the same.

Diagnostic efficacy of pancratic responses to the individual hormones was similar. An overall evaluation of the results seems to be the most reliable method for diagnostic purposes.

Keywords: synthetic secretin, cholecystokinin octapeptide, pancreatic function

Introduction

The secretin-pancreozymin test using different preparations of hormones is the standard method to detect pancreatic damage [1]. Recently, synthetic hormones have been available but their submaximal doses suitable for the secretin-pancreozymin test [12, 37] have not been precisely established. Neither is there any comparative study concerning the pancreatic response to synthetic secretin (S-S) and cholecystokinin octapeptide (CCK-OP) and their diagnostic efficacy in pancreatic disease.

In the present study we have examined the pancreatic response to S-S and CCK-OP in patients with pancreatic dysfunction and in control subjects to evaluate the diagnostic use of the synthetic hormones.

Materials and methods

Stimulation with increasing doses of secretin

Synthetic secretin was kindly provided by Prof. E. Wünsch (Max-Planck-Institut für Biochemie, München, FRG). It was lyophilized and taken up in a solution of cysteine-HCl

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in ampoules containing 50 μg of secretin and 1 mg of cysteine-HCl. Biological activity of the secretin preparation remained stable during this study and also for some weeks in 0.9% NaCl solution at —20 °C as controlled in rat experiments. (Recently, the synthetic secretin of Prof. Wünsch has been produced by Hoffmann—La Roche Co. Ltd., Basel, Switzerland.)

After simultaneous intubation of the stomach and the duodenum with a double lumen Balzer tube, the duodenal and gastric contents were aspirated in 10 min fractions with an electric pump. The basal fraction was discarded and increasing doses of 50–100–200 ng/kg of synthetic secretin were given to five volunteers in bolus injection. After each injection duodenal juice was collected for three successive 10 minute fractions. The volume and bicarbonate concentration of each sample were determined.

Stimulation with increasing doses of CCK-OP

CCK-OP was synthetized and biologically characterized by our team [18]. After intubation with the Balzer tube, to five other volunteers 25, 50 or 75 ng/kg of CCK-OP was given slowly i.v. to avoid the side effects produced by rapid injection. After each dose duodenal juice was collected for 10 min. The samples were put immediately in ice and examined within 4 h. Trypsin, lipase and amylase secretion was measured.

Secretin-cholecystokinin-octapeptide (S-S-CCK-OP) test

Duodenal and gastric juice were aspirated separately in 10 min fractions with the Balzer tube. The basal fraction was discarded and submaximal dose (125 ng/kg) of S-S was given i.v. as bolus injection and duodenal juice was collected for 30 min. Then a submaximal dose (50 ng/kg) of CCK-OP was given intravenously during 5 min and the duodenal contents were collected for 30 min. In the postsecretin phase some times gastric reflux occurred, so the loss of duodenal volume was corrected by estimating trypsin in the gastric aspirate. No other correction of volume recovery was used.

Chemical methods

Trypsin activity was estimated spectrophotometrically with α-N-benzoyl-D-L-arginine p-nitroanilide-HCl (BAPNA, Sigma) as substrate [3], lipase by a pH-stat method with Sigma lipase substrate [15] and amylase with the Phadebas® Amylase Test (Pharmacia AB, Uppsala, Sweden). Bicarbonate concentration was estimated by back-titration to pH 5 [3].

Calculations

Maximum and mean enzyme activity, and maximum and cumulative outputs for the 10 min periods were calculated for all the enzymes. Maximum and cumulative volumes and in the postsecretin phase also maximum bicarbonate concentration were registered for diagnostic purposes. Lower limits of normal values were determined with the formula, $\log \bar{x}$-2 log SD, [32] using data of 31 control subjects. The results were evaluated statistically by Student's *t* test or variance analysis whenever it seemed appropiated.

Material

The study was performed in 41 control subjects having no pancreatic disease and in 103 patients with pancreatic dysfunction.

The diagnosis of pancreatic disease was assumed on the basis of a typical history and the laboratory signs of two or more relapses of pancreatitis and by simple screening procedures such as the starch tolerance and/or the Lipiodol test [2] and later verified by Lundh test [25]. In 68 patients additional morphological examinations (histology and/or endoscopic wirsungography) proved the existence of chronic pancreatitis.

Results

50–100 ng/kg of S-S resulted in an almost dose-related increase in volume but larger doses caused a significant decrease in pancreatic volume in the first 10 min period and no further increase in the next two periods (Fig. 1). The bicarbonate response showed no significant change 25–50 ng/kg of CCK-OP stimulated enzyme secretion in a dose-related manner but subsequent injection of 75 ng/kg of CCK-OP caused not further increase or even inhibited lipase output (Fig. 2).

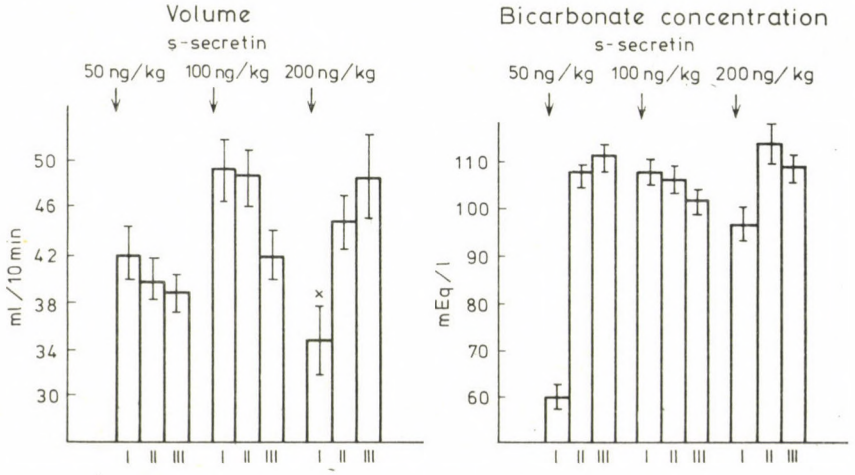

Fig. 1. Volume and bicarbonate response to increasing doses of synthetic(s) secretin. Duodenal juice was collected in three successive 10 min periods (I—II—III) after each dose of hormone. Values represent means ± S.E.M. of five volunteers. Significant decrease in the volume response to the largest dose of secretin during the first 10 min period is indicated by asterisk ($P < 0.05$)

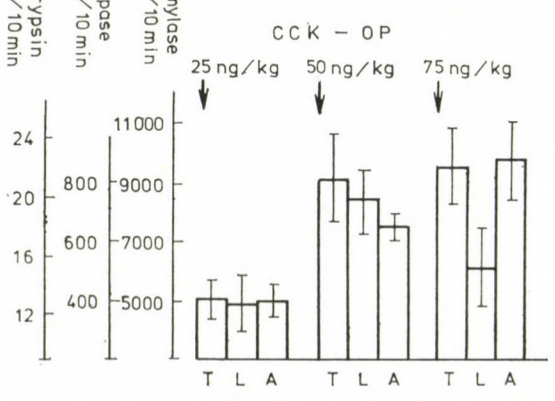

Fig. 2. Response to increasing doses of CCK-OP injection. Duodenal juice was collected for 10 min after each dose of hormone. Values are mean ± S.E.M. of five volunteers. T = trypsin, L = lipase, A = amylase

CCK-OP resulted in more trypsin but somewhat less lipase than did S-S; the response of amylase was similar to both hormones (Table I).

By the use of 14 parameters for each response of the patients, the frequency of positive results was less with the S-S test than with the CCK-OP or S-S-CCK-OP test. Maximum bicarbonate concentration gave additional

Table I

Pancreatic response to S-S and CCK-OP

	Control subjects (n = 31)		Patients (n = 103)	
	S-Secretin	CCK-OP	S-Secretin	CCK-OP
Amylase				
Mean activity, U/ml	177.46 ±16.760	186.11 ±11.884	104.23 ±10.104	104.48 ±9.490
Maximum activity, U/ml	271.37 ±26.591	242.65 ±15.099	155.72 ±14.104	139.64 ±12.206
Cumulated output, U/30′	24 987.88 ±2 876.519	25 670.41 ±2 317.514	10 140.04 ±1 133.602	10 625.39 ±1 122.569
Maximum output, U/10′	12 585.92 ±1 510.503	15 364.47 ±1 393.315	5 785.72 ±688.499	6 083.38 ±690.855
Trypsin				
Mean activity, mU/ml	324.98* ±34.625	499.36 ±45.595	215.11* ±14.410	315.54 ±18.730
Maximum activity, mU/ml	522.52* ±65.031	745.97 ±68.525	338.40* ±24.072	425.29 ±25.047
Cumulated output, U/30′	44.78* ±4.851	68.11 ±4.451	19.76* ±1.426	31.05 ±2.410
Maximum output, U/10′	25.25* ±3.260	45.07 ±3.600	11.55* ±0.868	17.88 ±1.543
Lipase				
Mean activity, U/ml	69.83 ±7.039	54.83 ±5.099	45.72* ±3.208	32.87 ±3.113
Maximum activity, U/ml	94.39* ±10.711	72.13 ±5.906	56.93* ±3.720	44.53 ±3.965
Cumulated output, U/30′	10 285.74* ±1 473.344	6 643.56 ±721.178	4 405.07* ±391.263	2 874.92 ±295.869
Maximum output, U/10′	4 549.06 ±611.887	3 293.45 ±350.547	2 021.86* ±170.701	1 407.02 ±134.056
Volume				
Cumulated, ml/30′	137.32 ±7.962	129.97 ±8.302	86.01 ±3.335	91.39 ±3.857
Maximum, ml/10′	55.26 ±2.855	65.58 ±3.976	38.56 ±1.585	46.36 ±2.285
Maximum bicarbonate concentration, mEq/l	109.35 ±2.715	—	88.26 ±2.971	—

Values are mean ± S.E.M.; * significantly different from CCK-OP result (variance analysis, $P < 0.05$)

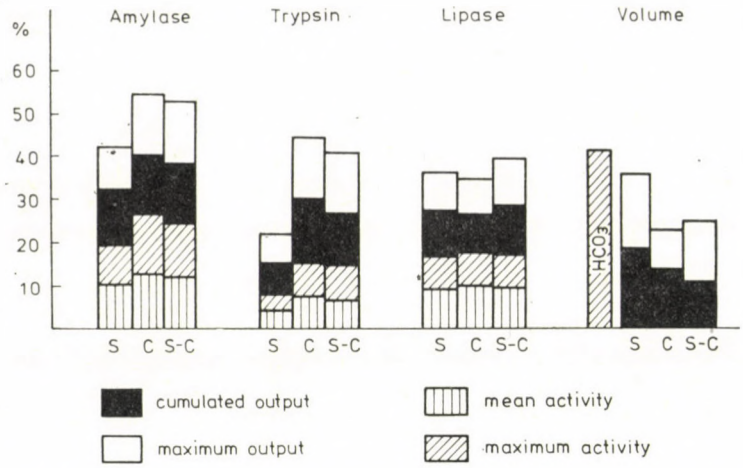

Fig. 3. Frequency of positive parameters of Synthetic Secretin-CCK-OP test in 103 patients
S = synthetic secretin, C = CCK-OP, S-C = overall response to S-CCK-OP

information only in the postsecretin phase (Fig. 3). In mild cases only individual enzyme activities or outputs, eventually only the volume outputs were pathological in one of the tests. The number of positive results increased parallel with the severity of pancreatic disease. False positive results were obtained in the control subjects with the S-S, CCK-OP and the S-S-CCK-OP tests at a frequency of 12.9%, 16.1% and 12.9%, respectively.

In histologically or endoscopically verified chronic pancreatitis, some false negative results occurred with all the three test at a frequency of 11.8%, 7.3%, and 7.3%, respectively, but one of them was always positive except in one patient.

The diagnostic efficacy [21] of the S-S and CCK-OP tests was similar and their sensitivity increased but the specificity decreased when both tests were evaluated. The most reliable results were achieved with the overall response to the S-S-CCK-OP test (Table II).

Table II

Diagnostic efficacy of S-S-CCK-OP test in chronic pancreatitis, per cent

	S-Secretin response	CCK-OP response	S-Secretin and /or CCK-OP response	Overall response to S-S-CCK-OP
Sensitivity	88.2	92.7	98.5	92.7
Specificity	87.1	83.9	71.0	87.1
Predictive value of positive test (true positive)	93.7	92.6	88.2	94.0
Predictive value of negative test (true negative)	77.1	83.9	95.6	84.4

Discussion

The total functional capacity of the pancreas characterized by its response to maximal stimuli points to the functioning cell mass of the organ [12, 37]. Supramaximal doses of S-S and CCK-OP, however, resulted in an inhibition of pancratic responses. Inhibition of secretion by supramaximal doses seems to be a general property of pancreatic secretion [27, 34], making it difficult to establish the exact doses needed for functional tests. In our study near maximal but evidently not supramaximal doses were used. The maximal dose of Squibb synthetic secretin was large for volume but not for bicarbonate concentration [11, 16], while a similar dose of caerulein was needed for both experiments [30]. Bolus injections instead of infusions of synthetic hormones were administered in view of the rapid inactivation of highly diluted preparations on different surfaces [4, 19].

The 125 ng/kg dose of S-S corresponding to about 0.5 CU/kg of GIH secretin [11, 13, 16] was comparable to the doses previously used for diagnostic purposes as submaximal stimuli [29, 37]. 50 ng of CCK-OP has a potency of 0.8 IDU according to animal experiments [23]. Similar doses of natural preparations [37] and synthetic caerulein [30, 33] were used in other human studies. The pancreatic response to Boots secretin (1 CHRU/kg) and pancreozymin (1.5 CHRU/kg) was similar but somewhat lower than in the control subjects of our previous study [26]. All measured and calculated parameters are needed to ensure the optimal diagnostic efficacy of the tests [9, 14]. By a complex evaluation of the secretory parameters it will be possible to correct the failure arising from the overlap of results of mild cases and of the controls. Discordant results are well-known to occur in mild or moderate pancreatic disease [20], even a negative S-P or Lundh test may be frequent in endoscopically verified chronic pancreatitis [22, 31] when few parameters are registered. Non-parallel disturbances of enzyme secretion may be the consequence of non-parallel enzyme synthesis and secretion [7] manifesting with pancreatic insufficiency shown by the data of less prevalent enzymes. The non-parallel inhibition of enzyme secretion by supramaximal doses of CCK [34] may be explained by the same mechanism. CCK-OP elicited more trypsin and about the same quantity of amylase than S-S in the control subjects as well as in the patients, also indicating the non-parallel secretion of pancreatic enzymes. The phenomenon was widely discussed and ascribed to differences in the acinar cell populations or to the presence of two intracellular enzymatic pools by Dagorn et al. [8]. The less pronounced increase in lipase secretion after CCK-OP may have been the consequence of a secondary inactivation of lipase activity by proteolytic enzymes [33] and/or of an increased bile acid secretion [15]. In any case, the washout enzyme secretion [5] in response to S-S was nearly as important as the direct stimulatory effect of CCK-OP.

Duodenal juice was aspirated by an electric pump and if the flow had decreased the patency of the tube was controlled by air-insufflation. In our preliminary study [28] no significant loss of juice to the intestine occurred, so the recovered volume of juice was not corrected. An uneven mixing of the marker in the intestinal contents leads to overestimation of the loss of juice [36] mainly in the postsecretin phase [5]. This may have been the reason why the secretory pattern and the volume responses corresponded to those obtained in other studies done without marker technique [6, 29, 37] but not to the corrected values of quantitative tests [5, 36].

The diagnostic efficacy of the CCK-OP tests was similar to that of the S-S test, although in the CCK-OP periods bicarbonate was not measured. In some studies using natural hormone preparations the postsecretin phase [10, 37], in others [6, 35, 37] the postpancreozymin period was more useful for the diagnosis of mild pancreatic disease. Using synthetic caerulein and natural secretin (GIH), the overlap of chronic pancreatitis and the control group was somewhat less wide with lipase and chymotrypsin output than with bicarbonate output [30]. In our further practice, the overall response to S-S+CCK-OP was calculated and used for diagnostic purposes since the greater sensitivity of the individually evaluated responses was deteriorated by their weaker specificity i.e. by their frequent false-positive results.

In conclusion, the diagnostic reliability of the S-S-CCK-OP test was equal to the secretin-pancreozymin test performed with natural hormones [24], but the latter procedure is less expensive than the former one and until now we have observed no allergic side effects which were rather frequent with the earlier preparations [6].

REFERENCES

1. Arvanitakis C., Cooke A. R.: Diagnostic tests of exocrine pancreatic function and disease. Gastroenterology **74,** 932–948 (1978).
2. Berger Z., Pap Á., Varró V.: Combined screening tests for pancreatic disease. Scand. J. Gastroent. **17,** Suppl. 78, 393 (1982).
3. Berndt W.: Klinisch-chemische Methoden zur Labordiagnostik von Pankreaserkrankungen. Med. Lab. **8,** 1772187 (1972).
4. Bitar K. N., Zfass A. M., Makhlouf G. M.: Binding of secretin to plastic surfaces. Gastroenterology **75,** 1080–1082 (1978).
5. Braganza J. M., Herman K., Hine P., Kay G., Sandle G. I.: Pancreatic enzymes in human duodenal juice, a comparison of responses in secretin pancreozymin and Lundh Brogström test. Gut **19,** 358–366 (1978).
6. Burton P., Evans D. G., Harper A. A., Howat H. T., Oleesky S., Scott J. E., Varley H.: A test of pancreatic function in man based on the analysis of duodenal contents after administration of secretin and pancreozymin. Gut **1,** 1112124 (1960).
7. Dagorn, J. C., Paradis D., Morriset, J.: Non-parallel response of amylase and chymotrypsinogen biosynthesis following pancreatic stimulation: a possible explanation for observed non-parallelism in pancreatic secretion. Digestion **15,** 110–121 (1977).
8. Dagorn J. C., Sahel J., Sarles H.: Non-parallel secretion of enzymes in human duodenal juice and pure pancreatic juice collected by endoscopic retrograde catheterization of the papilla. Gastroenterology **73,** 42–45 (1977).
9. Dobrilla G., Fratton A., Valentini M., Vantini I., Cavallini G., Angelini G., Mirachian R., Mora R.: Endoscopic retrograde pancreatography and secretin-pancreozymin test in diagnosis of chronic pancreatitis. A complex evaluation. Endoscopy **8,** 118–123 (1976).

10. Dreiling D. A.: Pancreatic secretory testing in 1974. Symposium on diagnosis of pancreatic disease. Gut **16,** 653–656 (1975).
11. Farooq O., Sturdevant R. A. L., Isenber J. I.: Comparison of synthetic and natural porcine secretins on human pancreatic secretion. Gastroenterology **66,** 2α4–2α9 (1974).
12. Grossman M. I.: Dose of secretin required for maximal response. Gastroenterology **60,** 157–158 (1971).
13. Gyr K., Kayasseh L., Rittmann W. W., Girard J., Stalder G. A.: Comparison of the biological potency of a new synthetic preparation of secretin with that of natural porcine secretin in the dog. Gut **19,** 355–357 (1978).
14. Hartley C. R., Cambill E. E., Engtröm G. W., Summerskill H. J.: Pancreatic exocrine function: comparison of response to augmented secretin stimulus, augmented pancreozymin stimulus, and test meal in health and disease. Amer. J. digest. Dis. **11,** 27–39 (1966).
15. Honegger J., Hadorn B.: The determination of lipase activity in human duodenal juice. Biol. Gastroent. **6,** 217–223 (1973).
16. Konturek S. J.: Pancreatic dose-response curves to intravenous secretin in man. Gastroenterology **58,** 828–832 (1970).
17. Lehnert P., Stahlheber H., Roder O., Zoelch M., Forell M. M., Wünsch E., Jaeger E., Marteus H. L.: Bestimmung der biologischen Aktivität von synthetischem Secretin. Klin. Wschr. **51,** 44–46 (1972).
18. Lonovics J., Pap Á., Penke B., Baláspiri L., Nárai Gy., Varró V.: Examinations on biological effects of the sulphate-ester of synthetic cholecystokinin-octapeptide. Kísérl. Orvostud. **30,** 404—413 (1978).
19. Lonovics J., Suddith R. L., Guzman S., Rayford P. L., Thompson J. C.: Loss of biologic and radioimmunologic activity of CCK-99% during sterilization by intracellulose filtration. New Engl. J. Med. **301,** 1066 (1979).
20. Lurie B., Brom B., Bank S., Novis B., Marks I. N.: Comparative response of exocrine pancreatic secretion following a test meal and secretin-pancreozymin stimulation. Scand. J. Gastroent. **8,** 27–32 (1973).
21. Masoero G., Andriulli A., Capussotti L., Ponzetto A., Lombardo L., Marchetto M., Arossa W.: Prospective evaluation of the diagnostic efficacy of a mathematical model in acute pancreatitis. Ital. J. Gastroent. **12,** 86–88 (1980).
22. Nakano S., Horiguchi Y., Takeda T., Suzuki T., Nakajima S.: Comparative diagnostic value of endoscopic pancreatography and pancreatic function tests. Scand. J. Gastroent. **9,** 383–390 (1974).
23. Ondetti M. A., Rubin B., Engel S. L.: Cholecystokinin-pancreozymin: recent development. Amer. J. digest. Dis. **15,** 149–156 (1970).
24. Otte M.: Pankreasfunktionsdiagnostik. Internist **20,** 331–340 (1979).
25. Pap Á., Berger Z., Varró V.: Trophic effect of cholecystokinin-octapeptide in man. A new way in the treatment of chronic pancreatitis? Digestion **21,** 163–168 (1981).
26. Pap Á., Berger Z., Varró V.: Összehasonlító vizsgálatok secretin-, pancreozymin-, és Lundh próbával. Orv. Hetil. **122,** 623–628 (1981).
27. Pap Á., Lechene de la Porte P., Sarles H.: Exogenous and endogenous stimulations during different phases of pancreatic secretion in conscious rats. Pflügers Arch. **383,** 19–27 (1979).
28. Pap Á., Nemessányi Z., Csernay L., Varró V.: Recovery rate of duodenal juice labelled with secreted $^{99}Tc^{m}$-HIDA during secretin-pancreozymin test. Digestion **25,** 59 (1982).
29. Petersen H.: The effect of pure natural secretin on the bicarbonate secretion into the duodenum in man. Scand. J. Gastroent. **5,** 105–111 (1970).
30. Ribet A., Tournut R., Diffaut M., Vaysse N.: Use of caerulein with submaximal doses of secretin as a test of pancreatic function in man. Gut **17,** 431–434 (1976).
31. Salmon P. R., Baddeley H., Machado G., Low-beer T., Rhys devies E., Trapnell J.: Endoscopic pancreatography, scintigraphy and exocrine function in pancreatitis. A comparative study. Gut **16,** 830 (1975).
32. Sarles H., Pastor J., Pauli A. M., Barthelemy M.: Determination of pancreatic function. Gastroenterologia Basel **99,** 279–300 (1963).
33. Vandermeers-Piret M. C., Vandermeers A., Rathé J., Christophe J., van der Hoeden R., Wettendorff P., Delcourt A.: A comparison of enzyme activities in duodenal aspirate following injection of secretin, cerulein and cholecystokinin. Digestion **10,** 191–204(1974).
34. Vaysse N., Laval J., Duffaut M., Ribet A.: Effect of secretin and graded doses of CCK-PZ on pancreatic secretion. Amer. J. digest. Dis. **19,** 887–894 (1974).
35. Wormsley K. G.: Response to infusion of a combination of secretin and pancreozymin in health and disease. Scand. J. Gastroent. **4,** 623–632 (1969).

36. Worning H.: Validity of polyethyleneglycol in the study of the total pancreatic secretion in man after stimulation by secretin, pancreozymin or food. Gut **8,** 599–604 (1967).
37. Zimmerman M. J., Dreiling D. A., Janowitz H. D.: The Secretin Test, Secretin, Cholecystokinin, Pancreozymin and Gastrin. Springer-Verlag, Berlin—Heidelberg—New York 1973, pp. 218–246.

Acta Medica Hungarica, Volume 40 (4), pp. 239–245 (1983)

LACTOSE-POOR MILK IN ADULT LACTOSE INTOLERANCE

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(Received June 11, 1982)

The frequency of lactose intolerance was studied in patients with chronic gastrointestinal disease, mainly peptic ulcer to explore the possibilities of the use of lactose-poor milk.

It was found that whole milk caused dyspeptic symptoms in 45% of patients with peptic ulcer; lactose-intolerance was present in 82% of the patients with milk intolerance; lactose poor powdered milk resulted in complete freedom from symptoms. Use of milk with reduced lactose contents is recommended in the diet of adult patients with lactase deficiency.

Keywords: lactose intolerance, peptic ulcer, lactose-poor milk

Introduction

Investigations over the last two decades have thrown new light on certain conditions connected with malabsorption or maldigestion and have transformed our views on the absorption and gastrointestinal effects of various foodstuffs. This allowed to detect an intolerance to some particular nutrient in the background of diarrhoea, abdominal colicks or maldigestion of seemingly obscure origin. It is no longer justified to ascribe conditions of this kind to "allergic gastroenteritis", since their cause may be entirely different. Conditions labelled "food hypersentivity" are often caused by milk or dairy products. Studies of recent years have shown that milk intolerance (MI) was generally caused by lactose maldigestion (LM) and consecutive lactose intolerance (LI) rather than by allergy to milk, and the factor responsible for the condition is an intestinal lactase deficiency (LD) [1, 2, 4–6].

Congenital lactase deficiency is a rare condition of recessive heredity, manisfesting itself with severe symptoms in the neonatal period.

Primary adult lactase deficiency is also hereditary hypolactasia, its incidence varying from population to population, in accordance with long-established dietary traditions [5, 6]. In the majority of the populations all over the world leaving the primitive pastorial tribes out of account, carriers of the lactase

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deficiency gene expressing itself after childhood with inadequate lactase activity of the intestinal brush border, may be estimated at 40 to 60%. This accounts for the excessively high prevalence of adult LI.

Secondary hypolactasia or alactasia, which may arise in children and adults alike as a result of some abnormal process involving the intestinal microvilli, is also fairly common. The responsible factors include intestinal infections, coeliac disease, gastrectomy or intestinal resection, and chronic inflammatory intestinal processes such as ulcerativ colitis, Crohn's disease and irritable colon. Chronic malnutrition and certain drugs like neomycin or cytostatics may also cause LD.

In LI, the spilling of lactose into monosaccharides is deficient, therefore the lactose is poorly absorbed and passes unaltered into the distal part of the small intestine and into the colon where, in consequence of its osmotic effect, it abstracts water from the body, thus providing a favourable medium for the growth of the ascending intestinal flora. Bacterial fermentation produces gases (H_2, CO_2, etc.) in abundance, as also various organic acids of low molecular weight which not only display an additional and strong osmotic effect, but also enhance peristalsis by acting as a chemical irritant to the intestinal wall.

These are the factors accounting for the symptoms of lactose maldigestion: diarrhoea, colicky pains, sensation of repletion, distention, flatulence (Fig. 1).

Management of LI was based earlier on complete abstinence from lactose. It is, however, difficult to keep a patient, particularly an infant, on a lactose-

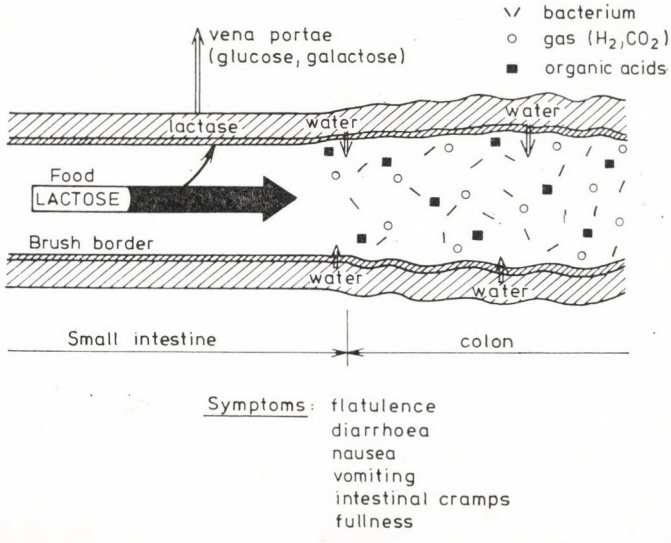

Fig. 1. Pathogenesis of the clinical features of lactase deficiency. Vena portae = portal vein

free diet without complete prohibition of milk. Also for otherwise healthy adults it is undesirable to be deprived from the benefits of milk and dairy products.

Until recently the dietary therapy of LM has been mainly of paediatric interest. Expensive, lactose-free foods (Lactopriv, Sojaval-G), or natural milk pretreated with beta-galactosidase (Galantase®) immediately before intake have been in use [2]. Home-prepared sour milk has been also used to good advantage [3].

Despite the wide range of possibilities, nutrition and dietary management of LI in children and adults, on account of the prevalence of LD in the population, still poses difficulties.

The present study was undertaken to establish the prevalence of LI by questionnaires; and to assess the clinical value of milks with reduced lactose content in the dietary management of LI.

Materials and methods

Data were collected by questionnaires directed at LI and its features from 150 outpatients with peptic ulcer attending regulary our clinic.

In 10 inpatients with peptic ulcer exhibiting symptoms of MI, oral loading with 1.5 g/kg lactose, was performed. Two days later the test was repeated with aqueous solutions of two different, but with regard to carbohydrate concentration identical, lactose-poor milk products made by the Hungarian Institute for Dairy Research:

1. Lactose-hydrolyzed milk powder obtained by beta-galactosidase (Maxilact-4000) pretreatment of skim milk at 35 °C for 3 h (Table I).

2. Ultrafiltered, lactose-poor powdered milk (Table II).

The oral test was repeated in the same patients with 1.5 g/kg of a mixture of glucose + galactose in equal proportion.

The glucose level was measured in capillary blood by an enzymatic assay (Boehringer Mannheim GmbH kit) in the fasting state and at 30 min intervals for 3 h after loading.

The subjective symptoms, the number of bowel motions, and the pH of the stool samples were registered.

LI was diagnosed if the increment of the blood glucose level in response to oral ingestion of lactose failed to exceed 1.0 mmol/l and the same time diarrhoea and other dyspeptic symptoms appeared.

For statistical analysis, Student's t test was used. The deviation of the means was regarded as significant at the level of $P < 0.05$.

In the diagrams the mean values $\pm$ S.E.M. are shown.

Table I

Compositon of lactose-hydrolized milk powder in g/dl

Galactose + glucose	51.4
Protein	36.0
Mineral salts	8.4
Water	3.5
Fat	0.7
Total	100.0

Table II

Composition of ultrafiltrated, lactose-poor powdered milk in g/dl

Glucose	37.1
Protein	28.7
Fat	19.0
Lactose	6.0
Water	5.9
Mineral salts	3.3
Total	100.0

Results

The questionnaires were returned by 120 out of 150 patients. 87 reported to be regular milk consumers. 54 of them (45%) experienced various symptoms after ingestion of milk. The symptoms, their types and incidence are listed in Table III. Some of the patients reported more than one symptom. Table III lists the essential symptoms only.

Thirty-three patients who had always avoided ingesting milk, the majority because of symptoms of dyspepsia.

Intolerance to milk has thus been reported by a large proportion of the aptients and it was attributed in the first place to intestinal LD.

Eight of the 10 closely investigated patients with active ulcer disease had duodenal, 2 had gastric ulcer. Their average age was 34.5 years, the male-to-female distribution 8:2.

Table III

Incidence of dyspeptic symptoms in milk-intolerant patients (n = 120) with gastric or duodenal ulcer

Main symptoms	No.	Per cent
Diarrhoea	16	21.7
Sensation of repletion	10	8.3
Colicks	8	6.6
Heartburn	5	4.2
Perspiration	3	2.5
Nausea	2	1.7
Total	44	45.0

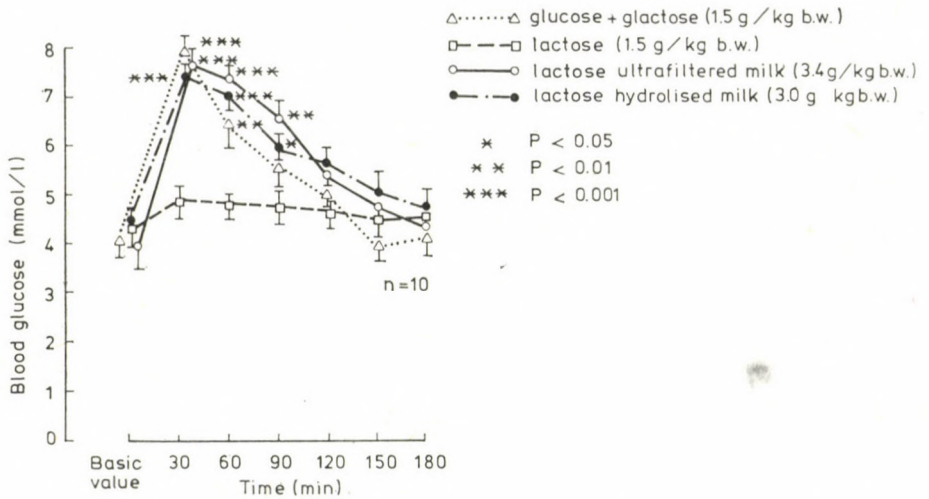

Fig. 2. Blood glucose level after loading with lactose, glucose + galactose,l actose-hydrolyzed and ultrafiltered milk products in patients with LI

The response of the blood glucose level to oral loading with lactose, subsequently with glucose+galactose and with lactose-poor milk are shown in Fig. 2. While the blood glucose level remained practically unchanged after lactose administration, it increased by 3.0 mmol/l after the other tolerance tests, reaching the peak at 30 min after ingestion.

The patients with LI developed watery stools and experienced abdominal colicks, a sensation of repletion and flatulence, often soon after lactose ingestion. The bowel motions varied between 2 and 12 in number. Faecal pH invariably

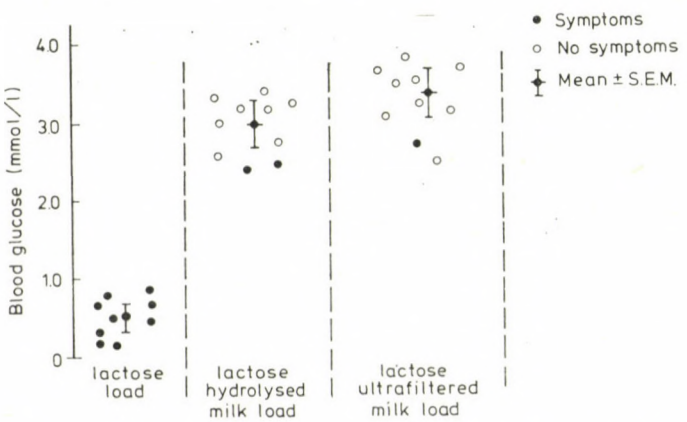

Fig. 3. Blood glucose level and dyspeptic symptoms after loading with lactose, lactose-hydrolyzed and lactose-ultrafiltered milk products (n = 10)

declined to figures of slight acidity. After ingestion of the lactose-poor milks these symptoms remained absent; only a minor distention was reported by one of the patients, and mild diarrhoea by another.

Discussion

The prevalence of MI is high in patients with gastrointestinal disease. Milk is an important dietary factor in some of these diseases, particularly in peptic ulcer, and is therefore, comsumed in large amounts. This discloses the presence of MI in many cases and accounts for its 45% prevalence in peptic ulcer.

We have confirmed the presence of lactose intolerance in 18 out of 22 patients (82%) with MI. This is consistent with published observations, according to which MI in adults may be attributed in practically all of the cases to LM of variable grades of severity resulting from intestinal LD [1].

It is not suggested that hypolactasia should be ascribed to peptic ulcer itself, although the prolonged dietary and long-term drug therapy may well have consequences of this kind. It is far more probable that hereditary intestinal LD involving a high proportion of the general population manifests in mature age primarily in patients with hyperacid gastritis or peptic ulcer as a result of excessive milk intake.

We still lack reliable data concerning the prevalence of LD in the normal adult population of Hungary. The proportion of 40% found in ulcer patients seems, however, to provide a fairly reliable estimate, which has yet to be confirmed by large-scale studies in normal populations.

The nutrition and diet of lactase-deficient adults still pose major practical problems. Whole-milk or food prepared with milk or dairy products are avoided by these patients. Their reluctance to it is justified. Milk which belongs to the essential elements of nutrition in our days is a source of abdominal discomfort, of dyspeptic symptoms in patients with LD. Withdrawal of milk offers an inadequate and at best a provisory solution.

In view of these problems lactose-poor milk is gladly accepted for clinical trials. These preparations adequately diluted in water, are in no way inferior in taste to skim milk.

Both lactose-hydrolyzed and ultrafiltered lactose-poor milks were subjected to comparative oral loading tests in 10 inpatients with peptic ulcer dysplaying symptoms of LI.

Intake of the prepared milk in a diluted form in quantities corresponding to fairly large volumes of natural milk caused no noteworthy symptoms of dyspepsia. At the same time, a marked elevation of the blood glucose level, as opposed to the lactose tolerance curves, was demonstrable in all of the cases

(Fig. 2). The response of the blood glucose level was consistent with the glucose + galactose tolerance curves.

On the evidence of the present study, lactose-poor milk preparations have thus been found suited for the dietary regimen in LD. Unless their cost should turn out to be prohibitive, they are bound to contribute to milk consumption. Selection of the biologically optimum products requires further experimental and clinical studies.

REFERENCES

1. Demény, S., Dán, S.: A felnőttkori lactose intoleranciáról. Orv. Hetil. **109,** 622–624 (1968),
2. Frank, K., Barna, M.: A másodlagos lactose malabsorptio enzymsubstitutios kezelése. Orv. Hetil. **118,** 2093–2095, (1977).
3. Jákics, J., Hernádi E., Breitner, V.: Lactose-mentes tejdiéta alkalmazása heveny és elhúzódó csecsemőkori hasmenések kezelésében. Gyermekgyógyászat **25,** 504–509 (1974).
4. Jávor, T.: Enzimdefektusok szerepe az anyagcsere betegségekben. Magyar Belorv. Arch. (in press).
5. Newcomer, A. D.: Milk, lactose and gene distribution. Amer. J. dig. Dis. **23,** 961–962 (1978).
6. Simoons, F. J.: The geographic hypothesis and lactose malabsorption. Amer. J. dig. Dis. **23.** 963–980 (1978).

Acta Medica Hungarica, Volume 40 (4), pp. 247—250 (1983)

THE EFFECT OF CYTOSTATICS ON THE INTESTINAL ABSORPTION OF D-XYLOSE IN PATIENTS WITH MALIGNANT LYMPHOMA

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(Received December 22, 1982)

The absorption of D-xylose was studied in patients with malignant lymphoma treated with cytostatics according to C. O. P. protocol. It was found that the absorption of D-xylose decreased for 24–48 h after the administration of the drugs. Following the cytostatics therapy a temporary arteficial malabsorption can be expected.

Keywords: citostatics, intestinal absorption, d-xylose, malignant lymphoma

Introduction

There is no tumour specific cytostatic drug available which would influence the tumour cells selectively. The sensitivity of certain tissues to cytostatics corresponds to the frequency of cell formation, thus the rapidly proliferating epithelia of the gastrointestinal tract can also be damaged during treatment [1, 3]. The xylose test is a simple way of judging intestinal absorption. On the basis of these we began to examine the effect of cytostatics on xylose absorption.

Patients and methods

Eleven patients with malignant lymphoma treated according to the C. O. P. protocol participated in the study. D-Xylose test was performed before and after 24–48 h of the combined cytostatic treatment. After the oral administration of D-xylose in 5 g doses urine was collected for 5 h in one h fractions. The result was given as g/5 h values. D-Xylose was determined by spectrophotometrical method. For the statistical analysis the Student's *t* test was used.

Results

The changes in the urinary excretion of D-xylose are shown in Fig. 1. There was no pathologically low excretion before the treatment. This means that D-xylose had been well absorbed from the intestine and excreted into the urine. After the administration of cytostatics a marked decrease occurred in xylose excretion on the first day, which improved on the second day. Figure 2 shows the means and the results of the statistical calculations. The

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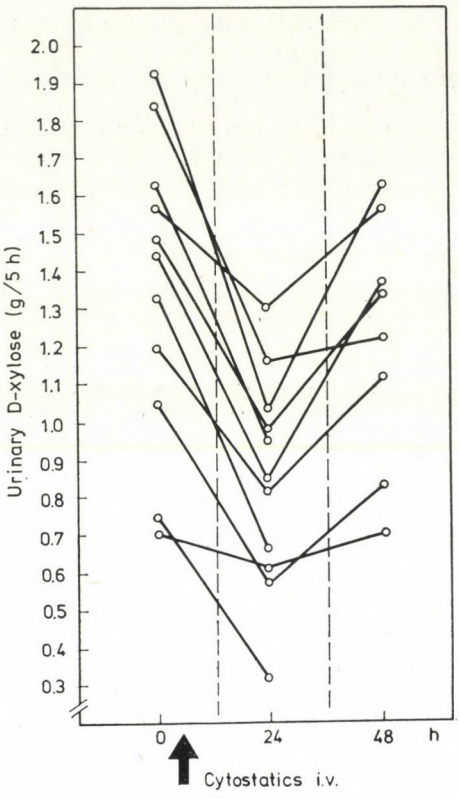

Fig. 1. D-Xylose excretion before and after combined cytostatic treatment. After the administration of cytostatics a marked decrease can be seen in xylose excretion on the first day, which improved on the second day

xylose values before the treatment were taken as 100%, and the results obtained on the first and second days were expressed as a per cent of the initial data. It can be seen that after the administration of cytostatics the decrease in xylose excretion is significant on the first and second days.

Discussion

Xylose is a five-carbon sugar that is absorbed by the same transport mechanisms as hexoses, but is characterized by a much slower rate than glucose or galactose. Assimilation of this sugar does not require the intraluminal pancreatic stage of digestion, and hence, impairment of xylose absorption indicates small intestinal mucosal disease. The xylose absorption test is simple, painless, free of risks for the patient and it can be repeated daily if necessary. It is the most reliable test of the functional capacity of intestinal absorption.

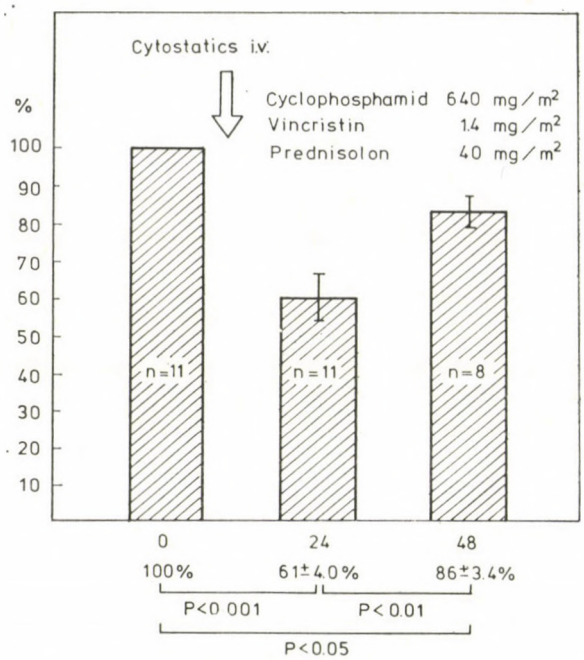

Fig. 2. Urinary D-xylose excretion before and after combined cytostatic treatment in percentage of the initial values (means ±S. E. M.). On the first day the change was significant, while on the second day it was quite moderate in comparison to the initial values

The following three mechanisms caused by cytostatics might be responsible for the changes observed:

1. Inhibition of mitosis (morphological changes)
2. Decrease of the activity of the mucosal enzymes
3. Shortened intestinal passage

Cytostatics act in various phases of the generational cycle of cells, and exert their effect by the way of inhibiting the division of cells [2, 7, 9]. This means that healthy tissues are involved in particular tissues that manifest a high degree of proliferation. The small bowel mucosa possesses the fastest mitotic activity after the haemopoietic system, therefore its functional and morphological damage during cytostatic treatment should be expected [1, 3, 6, 8]. Following high doses of cytostatic substances the mitotic activity of the cells is strongly reduced. On the first day after treatment, the small bowel mucosa, showed necrotic cells in the crypts and mitoses arrested in metaphase. By the second day severe villous atrophy and occasional erosions were seen.

In the literature many reports can be found on the activity of disaccharidases after the administration of cytostatics [4, 5]. The results are, however, not uniform. We could demonstrate earlier that certain cytostatics decrease

the enzyme activity of Na^+—K^+-dependent ATP-ase, which enzyme plays an important role in active transport processes [10].

Cytostatic treatment is often accompanied by diarrhoea resulting in a shortened intestinal passage.

Cytostatics seriously damage the small intestinal mucosa, the netto absorption decreases, and the activity of the enzymes decreases. The physicochemical characteristics of the glycocalyx may change. The damaged mucosa loses its barrier function and it can be supposed that these complex pathophysiological processes may also be responsible for the impaired D-xylose absorption.

Our final conclusion is that more attention should be paid to the possibility of a therapeutically induced malabsorption in patients under treatment with cytostatics.

REFERENCES

1. Beró, T., Molnár, L., Jávor T.: The effect of cytostatics on intestinal protein loss in rats. In: Mózsik, Gy., Hanninen, O., Jávor, T. (eds): Adv. Physiol. Sci. Vol. 29. Gastrointestinal Defence Mechanisms. Pergamon Press, Oxford and Akadémiai Kiadó, Budapest 1981. pp. 315–326.
2. Ecknauer, R.: Morphology and enzyme activity in rat small intestinal epithelium 6 and 12 hrs. after an alkylating agent (Cyclophosphamide). Acta Hepato-Gastroenterol. **23,** 365–371 (1976).
3. Hartwich, G.: Side effects of a cytostatic treatment on the gastrointestinal tract. Acta Hepato-Gastroenterol. **21,** 89–92 (1974).
4. Hartwich, G., Leicher, H., Müller, H., Domschke, W., Matzkies, E.: Disaccharidasen der Dünndarmschleimhaut der Ratte unter zytostatischer Behandlung mit Vincristinsulfat. Arzneim.-Forsch. (Drug Res.) **26,** 58–62 (1976).
5. Hartwich, G., Domschke, W., Matzkies, E.: Disaccharidasen der Dünndarmschleimhaut der Ratte unter zytostatischer Kombinationsbehandlung mit Vincristinsulfat und Ifosamid. Arzneim.-Forsch. (Drug Res.) **26,** 350–352 (1976).
6. Hobson, R. W., Jervis, H. R., Kingry, R. L., Wallace, J. R.: Small bowel changes associated with vincristine sulfate treatment: an experimental study in the guinea pig. Cancer **34,** 1888—1896 (1974).
7. Jellinghaus, W., Schultze, B., Maurer, W.: The effect of vincristine on mouse jejunal crypt cells of differing cell age: double labelling autoradiographic studies using 3-H- and 14-C-TdR. Cell Tissue Kinet. **10,** 147–156 (1977).
8. Neumeister, K., Beyer, W., Pfeiffer, J.: Side effects of chemotherapy of malignant tumors in the haematopoietic system and in the intestinal tract after preceded irradiation. Radiobiol. Radiother. (Berl.) **11,** 391–398. (1970).
9. Rybak, B. J.: Electron microscopic studies of intestinal lesions. I. Aminopterin-induced lesions in mice. Gastroenterology. **42,** 306–318 (1962).
10. Tárnok, F., Mózsik, Gy., Nagy, L., Jávor, T.: Effect of immunsuppresive drugs on active transport (Na^+–K^+-dependent) ATP-ase system from human gastric mucosa. In: Borsy, J., Mózsik, Gy. (eds): Gastrin and its Antagonists. Akadémiai Kiadó, Budapest 1971 .pp. 113–121.

Acta Medica Hungarica, Volume 40 (4), pp. 251—257 (1983)

EFFECT OF AMINOETHYL-ISOTHIURONIUM (AET) TREATMENT ON INTESTINAL CELL PROLIFERATION DURING AGEING

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To establish whether aminoethyl-isothiuronium . bromide . hydrobromide (AET) treatment influenced intestinal cell proliferation during ageing, juvenile and aged female rats were treated with AET for a period of 20 days. Subsequent incorporation of ^{3}H-thymidine was studied in the epithelial cells of the duodenal and jejunal crypts. It was found that after AET administration the major changes occurred in the duodenum, i.e. the number of the villous epithelial columnar cells were found to be elevated during senescence with a markedly higher level of mitotic numbers in the crypts. Changes were less in the ageing jejunum, here neither the cells nor the number of mitoses were as altered as those of the duodenal region. In general, the labelling indices of crypt cells increased in young ages after AET treatment. The findings can be explained by the role of AET with respect to nuclear metabolism.

Keywords: AET, cell proliferation, intestine, ageing

Introduction

Cell damage by bioreactive free radicals is one of the most important factors of ageing [9, 11, 13, 16]. It has thus been assumed that with advancing age radiation protective agents might influence the formation of free radicals. Therefore, we have studied the effect of AET (aminoethyl-isothiuronium. bromide. hydrobromide) one of the most efficient compounds acting on intestinal cell proliferation. As a model, we chose the proximal segment of the small intestine, partly because the cell proliferation rate is the highest in this region [2, 17] and partly in view of the increased accumulation of AET in the small intestine [3]. It seemed logical to assume that not only the cell damage induced by ionizing radiation can be reduced and cellular regeneration increased but the effect of free radicals might also be mitigated by the preventive administration of AET.

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Notations: Mitotic index (MI) was expressed as the percentage of cells in mitoses related to the total number of cells recorded. Percentage of labelled mitoses is related to the mitoses found in the crypts. (We considered a cell being labelled if the nucleus contained 4–5 silver grains.) Labelling index, LI, means the percentage of labelled crypt nuclei among the total cell nuclei.

Materials and methods

Thirty-two, 2 months old ($RLEF_1$ strain) female rats, weighing 153 ± 27 g and 32 aged (22–23 months old) animals weighing 276 ± 35 g, were used. The rats were maintained on standard laboratory chow (LATI, Gödöllő) and supplied with tap water ad libitum. The animals were given intraperitoneally 135 mg per 1000 g of body weight AET (aminoethyl-isothiuronium . bromide . hydrobromide, G. Richter, Budapest) dissolved in physiological saline adjusted to pH 7.3, in amounts of 1.0 ml/100 g body weight, every second day at 10.00 a.m. for a period of 20 days prior to radioactive labelling. ^{3}H-thymidine in a dose of 0.5 μCi/g (18.5 kBq) body weight (specific activity 22.3 Ci/mmol) (825.1 GBq); ÚVVVR, Prague) was injected intraperitoneally before starting the examination.

The rats were killed by decapitation at 1, 2, 4, 8, 16, 32, 64 and 128 h after thymidine administration. Portions (5 mm long) of the duodenum and jejunum were excised from the middle region distally to the pyloric–duodenal junction as well as distally to the ligament of Treitz, fixed in Bouin's fluid, washed, dehydrated and embedded in paraffin. Longitudinal sections, 5 μm thick, were cut and processed for autoradiography using Ilford K2 nuclear research emulsion in gel form (Ilford Ltd., Essex, England) diluted 1 : 1 with water. After exposition for 30 days at 4 °C the specimens were developed, fixed, washed, stained with haematoxylin–eosin and studied with an Opton microscope at × 490 magnification.

All counts were made on well oriented villus-crypt units cut axially and sampled according to the criteria of Cheng and Leblond [4, 5]. In these units the base and top were clearly discernible. Each of the groups included 16 animals. Five villus-crypt units were studied per animal and a total of 5120 data were recorded. The examinations were based on the number of epithelial columnar cells on the left-hand column of the villi and the proliferation compartment (lower two-thirds of the crypts) in conformity with Al-Dewachi et al. [1]. As regards the mitoses, only metaphases and anaphases were scored. The changes of the mitotic indices, the percentage of labelled mitoses and labelling indices were evaluated using Student's *t* test.

Results

Morphological changes of the villus-crypt units are presented in Fig. 1. The columns show the epithelial columnar cell number on the left-hand side of the villi and the number of crypt cells in the direct continuation of the villus concerned. The main changes were as follows. (*i*) In the old rats the number of

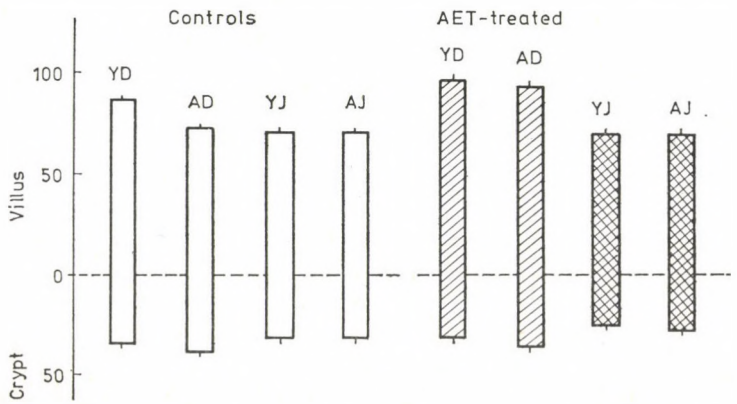

Fig. 1. Hatched columns mark the duodenal, and doubly hatched columns show the jejuna specimens of AET-treated animals. The ordinate shows the cell number of the villi and crypts. Verticals mean S. E. M. values (number of animals was 16 in every case). YD = young duodenum; AD = aged duodenum; YJ = young jejunum; AJ = aged jejunum

the epithelial columnar cells of the villi in the duodenum decreased significantly ($P < 0.01$) while the crypt cell number was increased ($P < 0.01$); (*ii*) the epithelial cell number of the duodenal villi was elevated in the AET-treated juvenile and old animals ($P < 0.01$); (*iii*) the number of crypt cells was usually unchanged or reduced after AET administration.

Table I shows the mitotic changes (indices) of the control and AET-treated animals. The data revealed that except in the young control and AET-treated jejunal specimens the mitotic indices were increased in almost every experimental group. The mitotic indices in the duodenal crypts of the juvenile rats were approx. half of those in the AET-treated young ($P < 0.01$) and the control and AET-treated aged rats ($P < 0.01$).

As concerns the percentage of labelled mitotic figures, all the animals showed significantly higher values in the 8 h period than in the 128 h period. Apart from one case (control aged duodenum), there was a discernible difference between the control and treated animals in the time-dependent reduction of

Table I

Effect of AET treatment on the cellular parameters of the duodenum and jejunum

Groups	Mitotic index		Percentage of labelled mitotic index		Labelling index	
	8 h period	128 h period	8 h period	128 h period	8 h period	128 h period
Controls, juvenile, duodenum	7.3^{1} ±0.3 (40)	7.5^{7} ±0.3 (80)	77^{15} ±3.4 (40)	65^{16} ±3.6 (80)	47^{31} ±2.1 (40)	49^{37} ±2.3 (80)
Controls aged, duodenum	8.0^{3} ±0.5	8.8^{9} ±0.4	82^{17} ±2.9	62^{18} ±3.5	66^{32} ±2.5	61^{38} ±2.1
Treated, juvenile, duodenum	11.6^{2} ±0.3	11.1^{8} ±0.3	75^{19} ±2.7	62^{20} ±2.8	63^{33} ±2.1	57^{39} ±1.8
Treated, aged, duodenum	12.4^{4} ±0.3	12.6^{10} ±0.3	75^{21} ±2.5	60^{22} ±3.3	63 ±1.5	58 ±2.0
Controls, juvenile, jejunum	12.3 ±0.8	10.3^{11} ±0.5	77^{23} ±2.8	66^{24} ±3.3	57^{34} ±2.2	55^{40} ±1.9
Controls, aged, jejunum	10.7^{5} ±0.7	10.0^{13} ±0.5	85^{25} ±2.9	72^{26} ±3.4	70^{35} ±1.9	67^{41} ±2.0
Treated, juvenile, jejunum	13.7 ±0.9	14.6^{12} ±0.6	86^{27} ±3.2	73^{28} ±3.3	70^{36} ±1.9	64^{42} ±1.8
Treated, aged, jejunum	12.9^{6} ±0.6	11.7^{14} ±0.4	84^{29} ±2.5	70^{30} ±3.6	63 ±1.9	56 ±2.0

Mean ± S.E.M. In parentheses, number of observations. The exponents denote the level of significance between the values: ($P < 0.05$); 5–6, 15–16, 23–24, 25–26 and 27–28; ($P < 0.01$); 1–2, 3–4, 7–8, 9–10, 11–12, 13–14, 17–18, 19–20, 21–22, 29–30, 31–32, 31–33, 34–35, 34–36, 37–38, 37–39, 40–41 and 40–42

the percentage of labelled mitotic figures. This would mean that the time-dependent decrease was more apparent in the treated than in the control animals. Simultaneously, the values were higher in the aged controls than in the young control. The difference in the percentage of labelled mitotic figures was less between the AET-treated aged and young rats than that of the percentage of labelled mitotic figures in the control aged and juvenile rats.

The labelling indices were significantly higher in the aged control animals, and considerably differences were observed in the duodenum and jejunum between the young controls and AET-treated young rats, i.e. the latter had appreciable higher labelling indices than the former ones.

Discussion

The morphological changes in the villus-crypt unit suggest that the functional villus cells play an important role in the regulation of crypt cell proliferation. The 15% decrease of the number of villus cells (cf. functional compartment) in the aged rats was accounted for in most cases by an expansion of the proliferative (progenitor) cell compartment in the crypts, particularly in the duodenum. It is believed that the reduction of the villus cell population affects the maturation of crypt cells perhaps as a result of the modification of cellular kinetics.

According to Lesher [14] the ageing process should be characterized individually for both the species and the strain concerned and it was emphasized that the cell population in the aged becomes more heterogeneous as time advances. The proliferative populations are completely asynchronous and the progenitor compartment shrinks in old age. Nevertheless, there are several compensatory reactions that allow to produce enough cells to replace the damaged ones and thus to maintain an appropriate functional compartment, i.e. the regular size of the villi. It must be added that the cells of the duodenal crypts are capable to speed up their generation time in both young and aged animals [14]. Hamilton [8] interpreted these processes as feedback mechanisms, which in young animals result in a rapid increase in cell production that soon returns to normal, after the progenitor compartment has become enlarged.

The present study allowed to conclude that the small gut is an organ sensitive to AET treatment. The increase of the cell number of the duodenal villi after AET treatment is strictly related to the increased mitotic activity (mitotic index) in the crypts of AET-treated animals.

Theoretically four factors could be responsible for the increase in the number of total mitoses, viz. (*i*) an increased number of cells in the M phase of the cycle; (*ii*) a reduced duration of the M phase; (*iii*) a combination of both these factors; and finally (*iv*) a reduced duration of the G_1 phase. This part of

the cell cycle is known to be the most variable one and believed to be undergo a considerable increase with age [14].

The per cent of labelled mitoses in the 8 h period in the duodenum and jejunum of non-treated aged animals proved to be higher than that of the young animals. This was mainly due to the longer generation cycle. On the other hand, the high percentage of labelled mitotic figures might be the consequence of the changed nuclear metabolism of the proliferation pool itself. If the percentage of labelled mitoses is related to the 128 h period, the values become less. This is particularly relevant in the case of the aged controls in comparison with the young untreated rats. The AET-treated young animals showed slighter reductions in the 8 and 128 h periods than the treated aged animals.

The increased labelling indices in the experimental groups may suggest that either the proliferative cell pool was augmented or the cell-cycle was prolonged, or both did occur. This assumption must, however, be quantified by considering the timing of cellular events together with the pool size of the whole proliferative cell compartment. From the present data it was not possible to establish such a relationship. Nevertheless, there are several factors which may account for the initially higher level of LI. Among these are the changing level of mitotic activity, the delayed or accelerated uptake of ^{3}H-thymidine and the shorter cell cycle duration. The rise in LI may be due to the division of labelled cells after the appearance of ^{3}H-thymidine followed by a retention of the resultant labelled daughter cells. Finally, elevation of the labelling indices in the experimental groups may be ascribed, partly, to an increased utilization of exogenous thymidine, and partly to the termination of mitoses in a part of cells due to the appearance of labelled daughter cells. One of the reasons of the marked uptake of the DNA label during the time spent with DNA synthesis by the treated animals is that the AET may somehow interfere with the metabolism of thymidyl nucleotides. AET would therefore appear to have mainly three perceptible effects in the intestine, i.e. on the number of epithelial cells, the mitotic indices and the labelling indices of the duodenal villi and crypts. However, the changes were not as apparent in the jejunum than in the duodenum. This was probably due to the poorer cell production in the jejunum which is, furthermore, more exposed to various structural changes than the proximal region of the small intestine [6]. These findings may also be explained by the altered structural differences in the villus/crypt unit of the jejunum. According to Fry et al. [6] the villus/crypt ratio in the duodenum was 10 against 7 in the jejunum.

Data are scarce concerning AET and cellular metabolism. According to the literature [7, 18] AET exerts a definite anticlastogenic effect in human lymphocytes. Vacek et al. [18] found in mice that the post-irradiation weight loss was considerably less after preventive AET treatment than in the untreated controls. At the same time after radiation injury the regeneration of bone mar-

row and splenic cells was enhanced by AET. Maisin et al [15] observed that the small intestine of AET-treated mice regained its weight after irradiation and this was due to an increase in the number of crypt cells. Our present data seem to agree with these observations.

It should be mentioned that in long-term experiments 2-mercaptoethanol, an analogue of mercapto-guanidine, the effective metabolite of AET, was found to prolong the lifespan of mice [10, 12]. This effect of the agent has been ascribed to its antioxidant action.

Acknowledgements

The authors wish to thank Dr. I. Perjés of Gedeon Richter Pharmaceutical Works Budapest, for the gift of aminoethyl-isothiuronium . bromide . hydrobromide and to Miss Magda Kelemen for skilful technical assistance.

REFERENCES

1. Al-Dewachi, H. S., Wright, N. A., Appleton, D. R., Watson, A. J.: The cell cycle time in the rat jejunal mucosa. Cell Tissue Kinet. **7,** 587–594 (1974).
2. Bhanthumnavin, K., Schuster, M. M.: Aging and gastrointestinal function. In: Finch, C. E. and Hayflick, L. (eds): Handbook of the Biology of Aging. Van Nostrand Reinhold Co., New York 1977. pp. 709–723,
3. Bradford, R. H., Shapira, R., Doherty, D. G.: Selective intracellular binding of radiation-protective agents by mammalian tissues. Fed. Proc. **16,** 157 (1957).
4. Cheng, H., Leblond, C. P.: Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. I. Columnar cells. Amer. J. Anat. **141,** 461–480 (1974).
5. Cheng, H., Leblond, C. P.: Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. V. Unitarian theory of the origin of the four epithelian cell types. Amer. J. Anat. **141,** 537–562 (1974).
6. Fry, R. J. M., Lesher, S., Kohn, H. I.: Age effect on cell-transit time in mouse jejunal epithelium. Amer. J. Physiol. **201,** 213–216 (1961).
7. Gebhart, E.: The anticlastogenic effect of various combinations of cysteamine, AET, HCT and amino acids on chromosome damage by Frenimon and bleomycin in human lymphocytes in vitro. Hum. Genet. **43,** 185–203 (1978).
8. Hamilton, E.: Cell proliferation and ageing in mouse colon. I. Repopulation after repeated X-ray injury in young and old mice. Cell Tissue Kinet. **11,** 423–431 (1978).
9. Harman, D.: Aging: a theory based on free radical and radiation chemistry. J. Geront. **11,** 298–300 (1956).
10. Harman, D.: Prolongation of the normal lifespan and inhibition of spontaneous cancer by antioxidants. J. Geront. **16,** 247–254 (1961).
11. Harman, D.: Role of free radical in mutations, cancer, ageing and the maintenance of life. Rad. Res. **16,** 753–764 (1962).
12. Harman, D.: Free radical theory of aging: effect of free radical inhibitors on the lifespan of male LAF_1 mice. J. Geront. **23,** 476–482 (1968).
13. Leibovitz, B. E., Siegel, B. V.: Aspects of free radical reactions in biological systems: aging. J. Geront. **35,** 45–56 (1980).
14. Lesher, S.: Chronic irradiation and ageing in mice and rats. In: Lindop, P. J. and Sacher, G. A. (eds) Radiation and Aging. pp. 183–206 Taylor and Francis Ltd., London 1966.
15. Maisin, J. R., Novelli, G. D., Doherty, D. G., Congdon, C. C.: Chemical protection of the alimentary tract of whole-body X-irradiated mice. 1. Changes in weight histology, and cell division in relation to nucleic acid and protein synthesis. Int. J. Rad. Biol. **2,** 281–293 (1960).

16. Miquel, J., Economos, A. C., Fleming, J., Johnson, J. E.: Mitochondrial role in cell aging. Exp. Geront. **15,** 575–591 (1980).
17. Moog, F.: The small intestine in old mice: growth, alkaline phosphatase and disaccharidase activities and deposition of amyloid. Exp. Geront. **12,** 223–235 (1977).
18. Vacek, A., Rotkovská, D., Sikulová, J., Dikovenko, E. A., Barkaja, V. S.: Protective effect of a mixture of radioprotective substances (AET and mexamine) on the haemopoietic stem cells of mice. Strahlentherapie, **134,** 424–429 (1978).

Acta Medica Hungarica, Volume 40 (4), pp. 259—270 (1983)

PENTAGASTRIN ANALOGUES CONTAINING α-AMINOOXY ACIDS

VII. Catabolism in vivo and in vitro of a pentagastrin analogue containing N-terminal aminooxyacetyl residue*

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In rat, BOC-NHO-1-^{14}C-Ac-Trp-Met-Asp-Phe-NH_2 (^{14}C-**I**) is metabolised more slowly than BOC-1-^{14}C-Gly-Trp-Met-Asp-Phe-NH_2 (^{14}C-**II**). Of the cytosol fractions of the rat organs the lung and pancreas exhibited a lower activity in the catabolism of BOC-NHOAc-Trp-Met-Asp-Phe-NH_2 (**I**) than in that of BOC-Gly-Trp-Met-Asp-Phe--NH_2 (**II**). In contrast, the cytosol fractions of the dog's lung, small intestine and pancreas hydrolysed **I** at a faster rate than they hydrolysed **II**.

Keywords: ^{14}C-labelled pentagastrin analogue: metabolism in vivo and in vitro

Introduction

Some pentagastrin analogues containing aminooxy acids gave rise in the rat to a greater acid secretion than that brought about by the reference pentagastrin [5]. We have demonstrated experimentally that tert-butyloxycarbonyl--aminooxy-acetyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalaninamide (BOC-NHOAc-Trp-Met-Asp-Phe-NH_2, **I**) and its L-α-aminooxypropionyl analogue remain in the rat's circulation for a longer time than does tert-butyloxycarbonylglycyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalaninamide (BOC-Gly-Trp-Met-Asp-Phe-NH_2, **II**) [7]. From this it was concluded that incorporation of the α-aminooxy acid increases the enzyme resistance of the analogues without influencing their biological activity (receptor binding). In the dog the biological activity of the analogues containing aminooxy acid does not attain that of pentagastrin [2, 2*a*].

In the present work we have studied the metabolism of labelled **I** in the rat, in order to obtain an answer as to its higher activity than that of **II**. It was

* For Part VI, see Ref. [1].

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further assumed that in the dog the lower biological activity of the analogues containing aminooxy acid is connected with their enhanced metabolism. To confirm this we have compared the proteolytic activities of the various organs to **I** and **II** in the rat and in the dog.

For the metabolic studies, ^{14}C-labelled **I** (BOC-NHO-1-^{14}C-Ac-Trp-Met--Asp-Phe-NH_2, ^{14}C-**I**) [1] and **II** (BOC-1-^{14}C-Gly-Trp-Met-Asp-Phe-NH_2, ^{14}C-**II**) [8, 9] were prepared in advance. It was confirmed by conductometric bioassay [1] that the labelling did not influence the biological activity of **I**. The metabolism of ^{14}C-**I** was compared with the earlier experimental results for ^{14}C-**II** [8, 9].

Materials and methods

The in vivo metabolism was studied in male Wistar rats weighing 200–250 g. ^{14}C-**I** with a specific activity of 18.43 MBq/mmol was injected intravenously into the rats in a volume of 1 ml/200 g body weight at a concentration of 0.4 μg/ml. Radioactivity was measured by a Nuclear Chicago ISOCAP-300 liquid scintillation spectrometer.

The catabolic activity of the various organs was examined in cytosol fractions obtained by ultracentrifuge sedimentation of the rat and dog organ homogenates. Cytosol fractions of the organs, and their mixtures with **I** or **II** in appropriate ratios, were incubated at 37 °C for 0, 30, 60, 90 or 120 min. The enzyme reaction was stopped with 0.2 mol/l EDTA. The immunoreaction **I** or **II** concentrations in the incubates were measured with the gastrin radioimmunoassay developed in our laboratory [4]. The antibody used for the radioimmunoassay was specific for amino acid sequence 13–16 of gastrin. Accordingly, **II** and **I** could be measured with 93% and 36.8% efficiency, respectively. The concentrations of the biologically active **I** and **II** were determined by means of conductometric bioassay [3].

Results

*Metabolism of ^{14}C-**I** in vivo*

Elimination of radioactivity from the circulation

Rats (6 animals on each occasion) were exsanguinated at various times after the intravenous injection of ^{14}C-**I** and the radioactivities in the sera were measured. With the fraction of the total activity injected per 1 ml serum plotted as a function of time, the elimination of radioactivity from the serum could be depicted graphically (Fig. 1).

Two straight regression lines could be fitted to the elimination curve. The half-lives calculated from the slopes of these regression lines were 1.4 min and 9.5 min.

The fact that the curve consisted of more than one section indicated that the activity was distributed between a number of compartments in the rat organism and that the activity measurable in the serum, except in the earliest stages, was the resultant of this multidirectional movement.

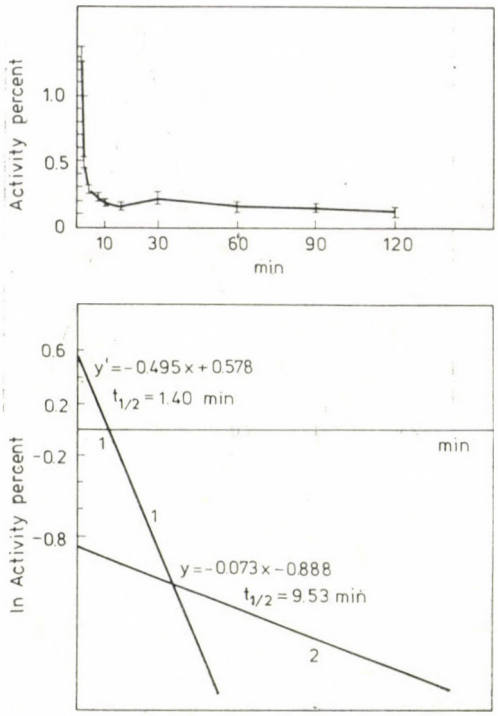

Fig. 1. Elimination of serum radioactivity

Composition of the serum radioactivity

To study the composition of serum radioactivity, blood was taken from the rat abdominal aorta 1 and 2 min after the intravenous administration of ^{14}C-**I** (5 rats on each occasion). The blood samples were centrifuged and 0.6 ml from each radioactive serum was chromatographed with 0.01 M phosphate buffer on a Sephadex G-25 fine column. The flow rate was 1 ml/min, with a column height of 38 cm, a diameter of 1.2 cm, $V_o = 16.6$ ml and $V_t = 39.8$ ml. Two ml fractions were collected and their radioactivity was measured. The radiochromatogram of the 1 min serum samples (the average radioactivity in dpm for the elution fractions) and the deviations for the samples are shown in Fig. 2.

Three radioactivity peaks were obtained in the chromatogram. The first of these (16–22 ml) had run with the serum proteins, the second (30–40 ml) corresponded to an unknown cleavage product of the ^{14}C-**I**, and the third (40–60 ml) was observed at the position for ^{14}C-**I**. The radioactivity of the cleavage product was almost the same as the radioactivity running with the serum proteins: 18.6 and 15%, respectively. The radiochromatogram of the 2 min serum samples resembled that of the 1 min samples, with the difference that

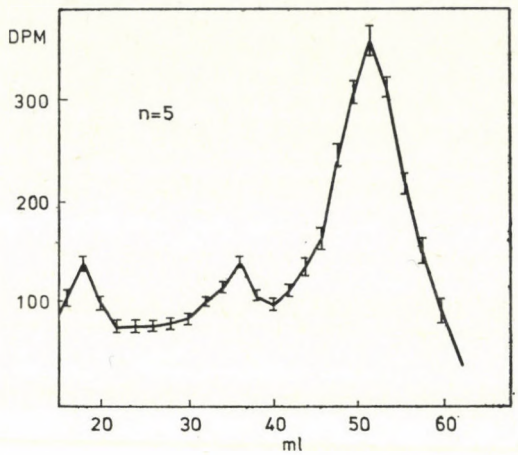

Fig. 2. Radiochromatogram of 1 min serum samples

the peptide-cleavage product had a higher activity (about 30% of the total activity eluted); the increase in activity of the cleavage product was accompanied by an equivalent decrease in the activity of ^{14}C-I; this supported our earlier assumption that the second activity peak originated from ^{14}C-I.

Elimination of the radioactivity by the liver

^{14}C-I was injected into the femoral vein of 5 rats and 20 min bile fractions were collected for 120 min through a cannula inserted into the common duct. The activities in the individual fractions, expressed as fractions of the total activity administered, were tabulated (Table I).

Table I

Mean ± S. D. of radioactivity of 30 min bile fractions

Animal No.	Fraction I	Fraction II	Fraction III	Fraction IV	Fractions I—IV
1	65.37	7.31	1.87	0.76	75.31
2	65.63	8.26	2.37	0.74	77.00
3	64.68	7.93	1.69	0.59	74.89
4	62.45	6.31	2.19	0.81	71.76
5	63.78	7.70	2.03	0.72	74.23
(X)	64.38	7.50	2.03	0.72	74.64
±SD	1.30	0.75	0.27	0.08	1.91

It may be seen from Table I that the activity was highest in the first fraction, with a subsequent progressive decrease in the further fractions. On the average, 74.6% of the total activity administered was excreted with the bile in 2 h.

Absorption of the radioactivity from the small intestine

Next, intestinal absorption of the radioactivity was studied. For this purpose, ^{14}C-I was injected in a dose of 1 ml/200 g body weight, at a concentration of 0.4 μg/ml, into the duodenum of 25 rats, and 5 animals each were exsanguinated 5, 10, 15, 30 and 60 min after the injection. Blood was taken simultaneously via cannulas inserted into the carotid artery and the portal vein. The activity percentages for the serum samples i.e. the activities of the samples taken at various times from the different vascular regions, as percentages of the total activity administered, and their mean values were plotted in a column diagram (Fig. 3).

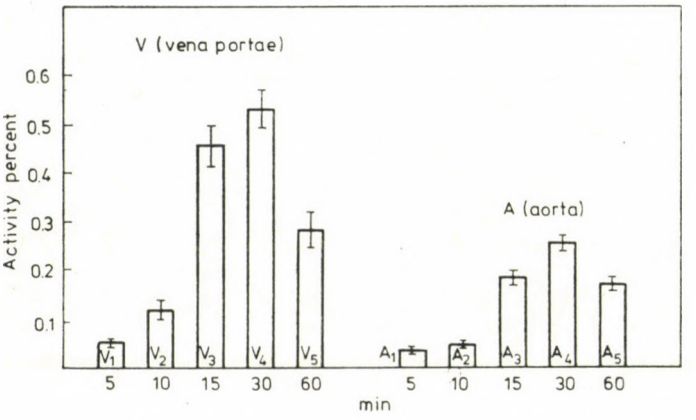

Fig. 3. Percentage radioactivity in serum obtained from aorta and portal vein after intraduodenal administration of ^{14}C-I

It may be seen from Fig. 3 that the activities of the serum samples obtained at 5, 10, 15 and 30 min from both the portal vein and the aorta increased gradually but there was a decrease in the 60 min sample as compared to the values after 30 min. The activity of the sera from the aorta was always lower than that originating from the portal vein.

Excretion of the radioactivity with urine and intestinal contents

The activity excreted with urine was examined after the injection of ^{14}C-I on one occasion into the tail vein of 6 rats placed in urine cages, and the activity of the urine excreted in 24 h was measured. 0.6 ml samples of the radioactive

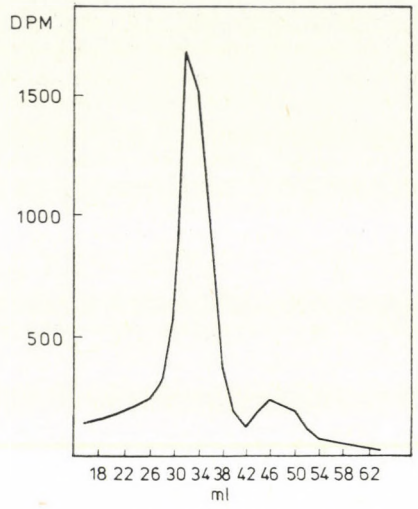

Fig. 4. Chromatogram of radioactivity excreted with the urine

urine were chromatographed on a Sephadex G-25 fine column. After 24 hours the animals were exsanguinated, and the activity of their small and large bowel contents were measured.

During 24 hours, an average of 39 $\pm$ 8% of the total activity administered was excreted with the urine, while 0.4% was found in the small intestine, and 1.3% in the large intestine.

A chromatogram of the activity excreted with the urine is shown in Fig. 4. This displays two peaks a larger (28–40 ml) eluted at the position of the radioactive peptide cleavage product, and a smaller (42–56 ml) at the position for ^{14}C-**I**.

Identification of the metabolite excreted with urine

For identification of the radioactive cleavage product, ^{14}C-**I** was injected into the caudal vein of 4 rats, and their urine was collected for 24 hours, concentrated to one-tenth and chromatographed on a Sephadex G-25 fine column. The fractions containing the cleavage product were collected, lyophilized and purified. The isolated product gave one radioactive spot on a thin-layer silica gel 60 chromatogram. The solvent systems were made by mixing ethyl acetate and a stock solution of pyridine/acetic acid/water = 20:6:11 in the following proportions: S1: ethyl acetate/stock 9:1, S2: ethyl acetate/stock 8:2, R_f^1 0.30–0.35, R_f^2 0.75–0.80; by its tlc behavior and its ^{1}H-NMR spectrum obtained by a JEOL-60 apparatus, this product was found to be identical with ^{14}C-**III** (tert-butyloxycarbonylaminooxy-1-^{14}C-acetic acid).

It could, therefore, be concluded that the rat has an enzyme that is able to cleave the CO-NH bond between the amino terminal aminooxy acid and the L-tryptophyl residue. As observed earlier [8] ^{14}C-**IV** (tert-butyloxycarbonylglycine) was formed from ^{14} C-**II** under similar conditions.

Organ distribution of the radioactivity

Finally, the organ distribution of the radioactivity was investigated 1 and 60 min after the intravenous injection of ^{14}C-**I**. Blood was taken from 5 animals at each of these times and the radioactivity was measured in the liver, kidney, lung, pancreas, jejunum and muscle tissue. These radioactivities were expressed as percentages of the total radioactivity administered per 100 mg wet tissue. The results are given in Table II, the values for each organ and each time being the means $\pm$ S. D. for 5 animals.

Table II

Distribution of radioactivity (mean $\pm$ S. D.) in the organs 1 and 60 min after intravenous administration of ^{14}C-**I**

	1 min	60 min
Liver	0.432 $\pm$ 0.083	0.051 $\pm$ 0.010
Kidney	0.277 $\pm$ 0.014	0.115 $\pm$ 0.027
Lung	0.043 $\pm$ 0.005	0.022 $\pm$ 0.002
Pancreas	0.029 $\pm$ 0.003	0.027 $\pm$ 0.003
Jejunum	0.049 $\pm$ 0.010	0.029 $\pm$ 0.002
Muscle	0.021 $\pm$ 0.002	0.018 $\pm$ 0.002

It may be seen from Table II that in the 1 min organ samples the highest activity was in the liver and the kidney. The activity in the other organs was approximately one order of magnitude lower. In the 60 min samples only the kidney showed an appreciable level of activity. Activity in he liver was much lower, but still evaluable while it was negligible in the other organs.

Study of **I** *and* **II** *catabolism in vitro by radioimmunoassay and bioassay*

The cytosol fractions of rat and dog organ homogenates were studied. Identical 2.0 μg amounts of **I** and **II** were incubated at 37 °C for 120 min with aliquots of the organ homogenates. The biologically active and immunoreactive peptide contents of the incubates were examined at 30 min intervals. The decrease in peptide concentration was plotted as a function of time. Figure 5 shows the changes in concentration of **I** and **II**, measured by radioimmunoassay

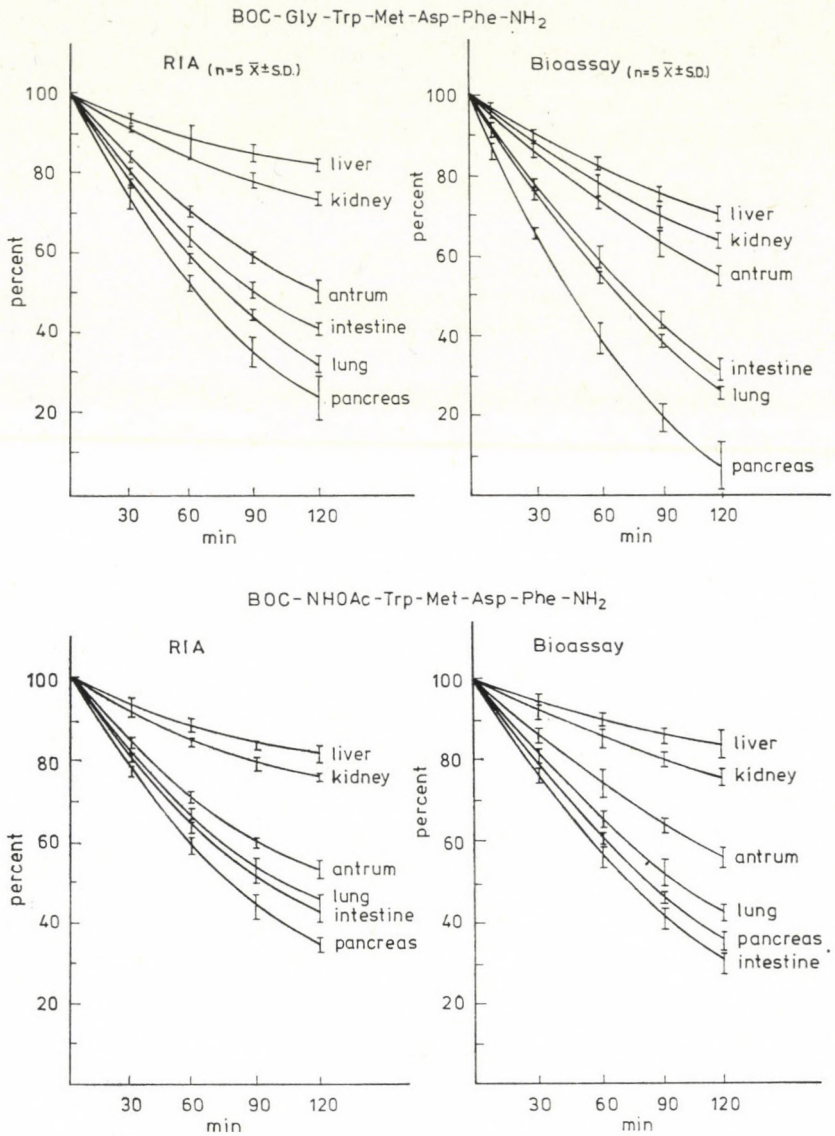

Fig. 5. Study of **I** and **II** catabolism with radioimmunoassay and bioassay. Enzyme activity of rat organs

and biossay, after incubation with cytosol fractions of rat liver, kidney, antrum, lung, intestine and pancreas specimes.

Figure 5 shows that the rate of catabolism of **I** (the slope of the decrease in the immunoreactive peptide concetration) was of the same order of magnitude as that of **II** after incubation with the liver and kidney cytosol fractions.

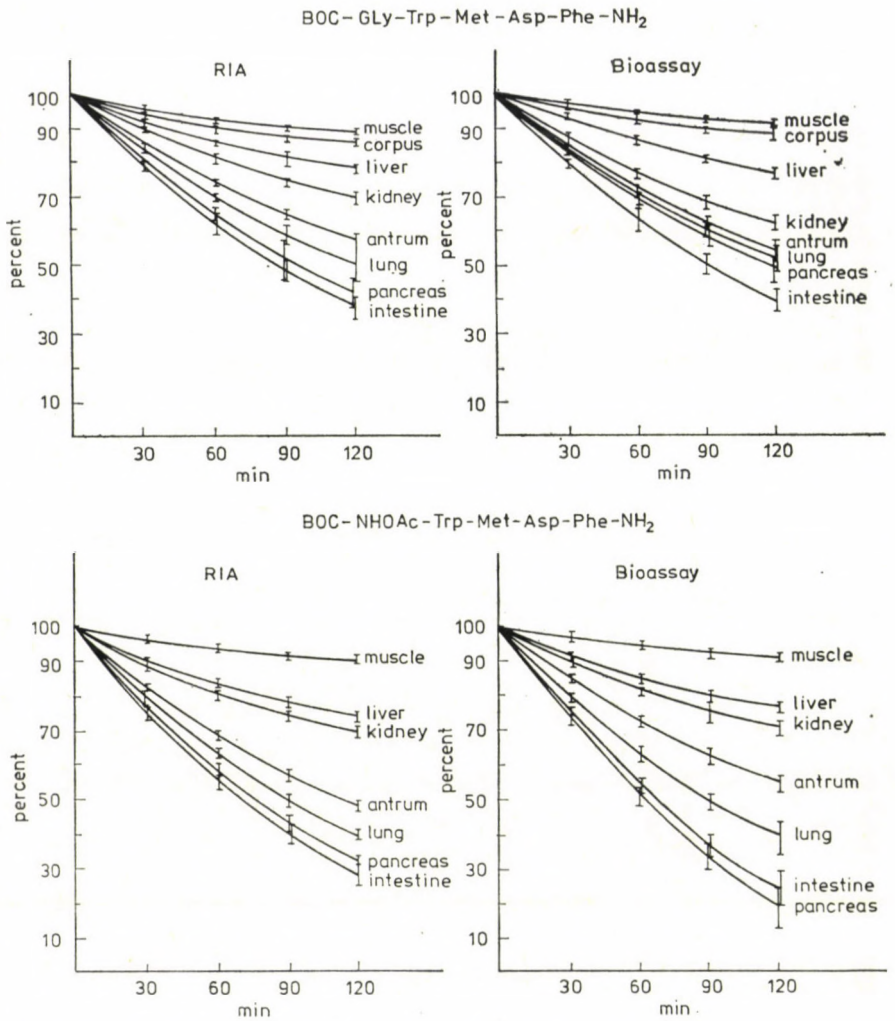

Fig. 6. Study of **I** and **II** catabolism with radioimmunoassay and bioassay. Enzyme activity of dog organs

Essentially the same was the case with the hydrolysing ability of the antrum and small intestine fractions. In contrast, the cytosol fraction of the lung and of the pancreas decreased the concentration of **I** at a lower rate than that of **II**. The difference in catabolism between the two peptides could be evaluated mathematically in both incubates: $P < 0.05$.

The change in the peptide concentration measured by bioassay went parallel with that of the immunoreactive peptide concentration in the course of incubation with the liver, kidney, antrum and lung cytosol fractions.

A small difference could be observed in the cases of the intestine and pancreas cytosol fractions. The decrease in peptide concentration measured on the basis of the biological effect in the mixture incubated with the intestinal fraction was somewhat larger, while that in the case of incubation with the pancreatic fraction was lower, than the corresponding concentration measured by RIA.

The peptide hydrolytic enzyme activities of the dog organs were studied with cytosol fractions of the liver, kidney, antrum, corpus, lung, small intestine, pancreas and muscle tissue. In this case too the peptide hydrolytic enzyme activities were characterized by a decrease in the immunoreactive and biologically active peptide concentrations (Fig. 6).

In the study of **I** catabolism the lowest enzyme activity was found in the muscle tissue and in the corpus mucosa. This was followed by the liver, the kidney and the antrum. The cytosol fractions of the lung, small intestine and pancreas tissue were markedly active. Comparing the catabolism of **I** to that of **II**, the muscle, liver and kidney hydrolysed the two analogues at approximately the same rate. Surprisingly, the cytosol fractions of the other organs hydrolysed **I** at a higher rate than they hydrolysed **II**. In the case of the lung, small intestine and pancreas, the difference in catabolism between the two peptides was mathematically significant, $p < 0.05$.

In 6 of the 7 organs examined, the decrease of the peptide concentration measured on the basis of the biological activity was parallel with that measured by RIA. A slight difference was found for the pancreatic cytosol: the peptie concentration measured by the biological activity decreased somewhat more than that measured by RIA.

Discussion

In the metabolism in vivo of **I**, in contrast with the metabolism of **II**, there was a striking difference in the radiochromatographic behaviour of the serum samples. Whereas the activity eluted with BOC-^{14}C-glycine ([^{4}C-**IV**) was the highest in the 1 min serum samples after the administration of ^{14}C-**II** and subsequently it decreased [8], the proportion of ^{14}C-**III** obtained after the administration of ^{14}C-**I** was lower in the 1 min samples than in the 2 min samples. If we add that the radioactivity of ^{14}C-**III** in the 1 min aortic blood samples was also lower than that of ^{14}C-**IV** after the administration of ^{14}C-**II** (18.6% and 27.5%, respectively [6], then we had to conclude from these data that less **I** than **II** is catabolised per minute in the rat.

Of the kinetic parameters of peptide metabolism, the elimination of serum radioactivity does not represent the disappearance of the peptide, earlier observed [8] to occur comparatively quickly, with a half-life of about 1 min. The liver cells secrete both **I** and **II** [10], but at the same time the radioactivity of ^{14}C-**III** originating from ^{14}C-**I** is absorbed across the intestinal wall

and excreted with the urine more slowly than in the case of ^{14}C-**IV** after the administration of ^{14}C-**II** [8, 9].

Chromatographic investigation of the activity excreted with urine provided a possibility for the isolation of the peptide cleavage product and the clarification of its chemical structure. It emerged from these investigations that the vast majority of the activity excreted with urine originates from the aminoterminal ^{14}C-**III** of ^{14}C-**I**. Thus, peptide hydrolysis took place between the tryptophan and the aminooxy acid connected to the BOC group.

The organ distribution of the activity indicates that the activities of both the 1 min and the 60 min samples point to the uptake of activity by those organs (liver, kidney) which take part in the elimination of the radioactive peptide or peptide fragment. The activity in the other organs remained low in both samples. The cause of this may be that ^{14}C-**III** cannot participate in the intermediate metabolism.

The studies suggested that in the rat ^{14}C-**I** is catabolized more slowly than is **II**. This observation, in conformity with the prolongation of the circulation time of derivatives containing aminooxy acid, may explain the increase in biological activity. These investigations did not however, reveal which are the organs to the enzyme activities of which the pentagastrin derivatives containing aminooxy acid become increasingly resistant. We have attempted to answer this question and that of the cause of the different strengths of the biological effect in the dog and the rat, on the basis of the concentration changes in the immunoreactive and biologically active **I**. The peptide hydrolytic enzyme activity in the rat and dog organs is completely different. Whereas the cytosol of the rat pancreas and lung hydrolysed **I** less than it did **II**, in dog the situation was the opposite. Of the dog organs, the enzymes of the gastric antral mucosa, the small intestine, the lung and the pancreas hydrolysed **I** to a greater extent than they did hydrolyse **II**. The difference in catabolism between the two analogues might explain the difference in biological activity observed in the rat and the dog.

REFERENCES

1. Baláspiri, L., Kovács, L., Schön, I., Kisfaludy, L., Kovács, K., Varga, L., Varró, V.: Pentagastrin analogs containing alfa-aminooxy acids. VI. Synthesis of two pentagastrin analogs ^{14}C-labelled at the N-terminal residue. J. Lab. Comp. and Rad., **9**, 469–77 (1982).
2. Bálint, G. S., Náfrádi, J., Kisfaludy, L., Schön, I., Varró, V.: Study of the structure-activity relationship in the case of various pentagastrin analogues. Acta physiol. Acad. Sci. hung., **53**, 113–114 (1971).
2a. Bálint, G. A., Náfrádi, J., Varró, V., Schön, I., Kisfaludy, L.: Pentagastrin analogs containing alfa-aminooxy acids, VIII. Biological studies in dog. Acta medica (submitted for publication).
3. Halter, F., Kohler, B., Smith, G. M.: A sensitive bioassay for gastrin. Helv. med. Acta Suppl., **50**, 113–114 (1971).
4. Remák, G., Penke, B., Német, J., Baláspiri, L., Kovács, L., Varga, L.: A 15-Leu-Humángasztrin 1–17 radioimmunoassay kidolgozásával szerzett tapasztalataink. Izotóptechnika (Budapest), **23**, 266–73 (1980).

5. Schőn, I., Kisfaludy, L., Náfrádi, J., Varga, L., Varró, V.: Pentagastrin analogs containing alfa-aminooxy acids III. Biological studies and structure-activity relationships. Hoppe-Seylers Z. physiol. Chem. **359,** 917–22 (1978).
6. Varga, L.: Catabolic pathways of the C-terminal pentapeptide of gastrin (pentagastrin) Advanc. physiol. Sci., 12, Digestion and Metabolism (Eds: T. Gáti, L. G., Szollár, Gy Ungvári) pp. 391–399, 1981.
7. Varga, L., Holzinger, G., Varga, E., Varró, V., Schőn, I., Kisfaludy, L.: Comparative study of pentagastrin analogues in the rat. Secretory response and elimination from the circulation. Acta med. Acad. Sci. hung., **38,** 77–80 (1981).
8. Varga, L., Sasvári, M., Penke, B., Baláspiri, L., Varró, V.: Metabolism of C-terminal pentapeptide of gastrin in the rat. Part III. The catabolism of the BOC-pentapeptide and its distribution in the organs. Acta hepato-gastroent. **26,** 499–503 (1979).
9. Varga, L., Sasvári, M., Penke, B., Baláspiri, L., Varró, V.: Metabolism of C-terminal pentapeptide of gastrin in the rat. Part IV. Catabolic pathway of the N-terminal BOC-^{14}-glycin fragment. Hepato-Gastroenterology, **27,** 146–59 (1980).
10. Varró, V., Varga, L., Csernay, L., Náfrádi, J., Penke, B., Baláspiri, L.: Hepatic and mesenteric metabolism of C-terminal pentapeptide of gastrin in the rat. Part I. Biliary excretion of labelled glycine-pentapeptide. Acta hepato-gastroent. **20,** 507–12 (1973).

Acta Medica Hungarica, Volume 40 (4), pp. 271—276 (1983)

Pulmonology

FIFTEEN YEAR FOLLOW-UP OF LUNG FUNCTION IN OBSTRUCTIVE AND NON-OBSTRUCTIVE PULMONARY TUBERCULOSIS

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(Received December 23, 1982)

Pulmonary function (VC, FEV_1, RV) was assessed in patients with pulmonary tuberculosis discharged in 1958/59 as curred or markedly improved. According to FEV_1/VC% the patients were divided into 40 obstructive and 59 non-obstructive cases and the tests were repeated by the same method in 1974. The control included physical and X-ray examinations, a questionnaire and the assessment of airway resistance (R_t).

In the non-obstructive group the change in VC in 15 years was only −27.7 ml/year, in the obstructive group, −54.3 ml/year. There was little difference between the two groups in the diminution of FEV_1 (28.8 and 35.3 ml/year, respectively). The material was subdivided into surgically and drug treated groups and studied statistically. The only significant change of function in the surgically treated group was in RV, whereas in the other group nearly all of the functional changes were significant statistically.

Keywords: pulmonary function, cured tuberculosis, obstructive syndrome, longitudinal study

Introduction

Reports published in recent years have shown many new results and it appears that in the much debated question of COLD (chronic obstructive lung disease) they have led to somewhat contradictory conclusions. Some authors observed a rapid decline in the functional and clinical condition of severe COLD patients with a very high mortality rate which tended to show an increase with the passage of years [3, 4, 5, 7, 8, etc.]. According to other authors in the milder cases the airway obstruction and the clinical condition of some patients showed little deterioration over periods of several years [1, 2, 6, 9, 11]. On the other hand they hardly include cases where the author was able to follow the changes in pulmonary function for more than 10 years.

Patients and methods

Patients with tuberculosis discharged as cured or in a considerably improved condition in 1958/59 were subjected to spirometric (VC, FEV_1, FEV_1/VC%) and residual volume (RV) determinations with the helium dilution method (Pulmotest and Pulmo-Analysor, Godart) a few weeks before they had left the Centre.

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The same tests were repeated in 1974 in identical circumstances and with the same equipment, and the patients were subjected to physical check-up, X-ray examination and body plethysmography.

The 1958/59 tests showed 40 patients to belong to the obstructive group ($FEV_1/VC\%$ below 70%) and 59 to the non-obstructive group. Of the originally obstructive patients 28, and of the non-obstructive patients 31 had been cured by drug treatment alone, without any surgical intervention and any pleuritic complication. The rest, i.e. 12 obstructive and 28 non-obstructive patients had been cured by surgical intervention or they had developed pleurisy with effusion during or prior to treatment. The surgical interventions or complications in the 12 obstructive cases were, segmental resection in 1, lobectomy in 4, pneumonectomy in 1, extrapleural pneumothorax in 1, pneumothorax in 4, and pleurisy with effusion in 1 case. In the 28 non-obstructive patients segmental resection was done in 4, lobectomy in 11, pneumonectomy in 3, thoracoplasty in 1, extrapleural pneumothorax in 4, intrapleural pneumothorax in 4 cases and one patient had a pleurisy with effusion.

The tests took place at least 2 months but usually 6 months to 2 years after the surgical intervention or pleurisy.

In most cases data on *smoking* were also obtained. The obstructive group included 25 smokers (67.5%) and 12 non-smokers; in the non-obstructive group there were 27 smokers (46.5%) and 31 non-smokers. These results have been published [13].

Sex: ten of the obstructive cases (25%) and 23 (39%) of the non-obstructive cases were women, 30 and 36, respectively, were men.

Age: mean age in the non-obstructive group at the beginning of the study in 1958/59 was 30.5 years and in the obstructive group, 40.4 years.

At the control examination, clinical symptoms indicative of *chronic bronchitis* were found in 15 obstructive cases (37.5%) and 12 non-obstructive cases (20.3%) [12].

Results

Results and the functional changes occurring during 15 years are shown in Fig. 1. In the *non-obstructive* cases the TC diminished by 11 ml, the VC by 416 ml, the FEV_1 by 432 ml, while the RV value showed an average increase of 404 ml. The *obstructive* cases had initially a higher RV and this increased by a further 671 ml during the 15 years. On the other hand, in the same cases TC diminished by 155 ml, VC by 815 ml, and FEV_1 by 530 ml. Since some of the cases had

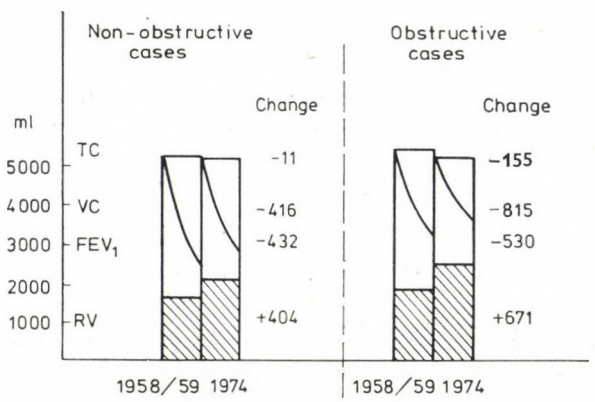

Fig. 1. Change of pulmonary function in 59 non-obstructive and 40 obstructive patients over 15 years

thoracic surgery before the tests, or pleurisy which must have considerably affected the pulmonary function (10, etc.) the functional changes in the surgically treated patients and those with pleurisy and the non-surgically treated cases will be examined separately.

Table I

Group	No. of cases			
	1958/59	1974	Changed to	
			obstructive	non-obstructive
NON-OBSTRUCTIVE				
Cured without surgery or complications	31	23	8	
with surgery or complications	28	22	6	
All cases	59	45	14	
OBSTRUCTIVE				
Cured without surgery or complications	28	25		3
with surgery or complications	12	8		4
All cases	40	33		7

It appears from Table I that of the 40 cases found to be obstructive in 1958/59, in 7 patients (3 non-surgically and 4 surgically treated) the respiratory channels had ceased to be obstructed in 1974, while from 59 non-obstructive cases 14 (8 surgically and 6 non-surgically treated) developed obstruction during the 15 years. Their detailed functional results have already been published [14]. Table II shows the functional changes in the obstructive and non-obstructive cases evaluated statistically by Student's *t* test and broken down into drug treated cases without pleuritic complication and cases where surgery had been performed or pleurisy had occurred. In the surgically treated non-obstructive patients the changes during the 15 years were not significant in any of the functional values, while in the other group the VC, FEV_1, $FEV_1/VC\%$, RV and RV/TC% showed statistically significant changes. In the group with obstruction the changes in the functional values that occurred in 15 years were significant statistically with the exception of the $FEV_1/VC\%$ in the drug treated group, while in the surgically treated or pleurisy complicated group only the increase in RV was significant.

Table II

Group		Average pulmonary function values									
		VC ml	P <	FEV_1 ml	P <	FEV_1/VC%	P <	RV ml	P <	RV/TC%	P <
NON-OBSTRUCTIVE											
Cured											
without surgery	1958/59	3888	0.05	3095	0.02	79.5	0.05	1617	0.001	28.3	0.001
or complications	1974	3490		2663		74.9		2310		37.4	
	Change	—398		—432		—4.6		+693		+9.1	
with surgery	1958/59	3134	0.20	2505	0.10	79.7	0.20	1715	0.7	35.6	0.10
or complications	1974	2700		2073		77.2		1799		39.—	
	Change	—434		—432		—2.5		+84		+3.4	
OBSTRUCTIVE											
Cured											
without surgery	1958/59	3770	0.001	2217	0.001	58—	0.3	2000	0.01	33.2	0.001
or complications	1974	2809		1520		53.8		2821		48.2	
	Change	—961		—697		—4.2		+821		+15.—	
with surgery	1958/59	2790	0.2	1810	0.3	64.—	0.3	1399	0.3	33—	0.001
or complications	1974	2314		1579		66.6		1686		42.2	
	Change	—476		—391		+2.6		+287		+9.2	

The *airway resistance* (R_t) determined in 1974 by body plethysmography amounted to 4.7 cm $H_2O/1/s$ in the obstructive group, and to 2.1 cm $H_2O/1/s$ in the non-obstructive group.

Discussion

In cured patients with obstructive and non-obstructive tuberculous affection we were able to trace the changes over a period of 15 years in some pulmonary function tests. Although COLD patients may include patients with tuberculosis cured some time earlier, the material cannot be regarded as identical with the patients with non-specific chronic bronchitis, asthma or emphysema. The material may also be influenced by the fact that most of the severely affected or old patients and those with serious functional defects had died or become unsuitable for out-patient control examinations during the 15 years. The main advantage and value of our material lies in the long period during which the patients' pulmonary function was observed and examined with identical methods. Comparing the changes in the 59 non-obstructive and 40 obstructive cases, we found that in the non-obstructive cases the decrease in vital capacity was remarkably low (27.7 ml/year); in the obtructive cases it was much higher (54.3 ml/year). The annual decrease in FEV_1 was very small and almost identical in the two groups (28.8 ml and 35.3 ml/year, respectively). The latter value appears to be particularly low if we take into account the high average age in the obstructive cases (55.4 years in 1974). There was a small difference in the increase in RV (26.9 ml and 44.7 ml/year, respectively), although in the obstructive group the increase was much more distinct than in the non-obstructive group.

In the surgically treated group the functional changes during the 15 years were so small that with the exception of the RV/TC% in the obstructive cases, they may be regarded as insignificant. This appears to prove that in 15 years survivors the operation had a minimal deteriorating effect on the pulmonary function or although it may have caused a certain functional damage, this did not deteriorate or even improved in the course of time. The very small increase in the residual volume in the surgically treated patients was particularly striking.

In contrast, the functional deterioration of the 31 non-obstructive and 28 obstructive cases treated only by drugs was significant in both groups with the exception of $FEV_1/VC\%$, precisely in the obstructive cases (Table II); there was however a slight difference between the two groups. Thus, contrary to expectations, the decrease in vital capacity in 15 years in the obstructive cases was significantly greater than in the non-obstructive cases ($P < 0.001$), while the decrease in the FEV_1 value did not differ significantly in the two

groups ($P < 0.1$) although it was higher in the obstructive group. Neither was the difference in the changes of RV significant.

In view of the above results, the question arises whether it is not misleading in many cases to regard the changes in $FEV_1/VC\%$ as a measure of the deterioration in ventilatory obstruction, since in the course of time the vital capacity decreases more than the FEV_1 if the symptoms of chronic bronchitis are not in the foreground. Thus the decrease, i.e. the deterioration, in the restrictive factor, (the vital capacity in the denominator) apparently improves the obstructive syndrome.

REFERENCES

1. Bates, D. V.: The fate of chronic bronchitis: A report of ten-year follow-up in the Canadian Department of Veterans Affairs coordinated study of chronic bronchitis. Amer. Rev. resp. Dis. **108,** 1043–1065 (1973).
2. Boushy, S. F., Thompson, H. K., North, L. B., Beale, A. R., Snow, R. T.: Prognosis in chronic obstructive pulmonary disease. Amer. Rev. resp. Dis. **108,** 1373–1383 (1973).
3. Burrows, B., Earle, R. H.: Prediction of survival in patients with chronic airway obstruction. Amer. Rev. resp. Dis. **99,** 865–871 (1969).
4. Burrows, B.: Prognosis of the bronchial and emphysematous types. In: Schüren, K. P., Hütteman, U., Schröder, R. (eds): Chronisch obstructive Lungenerkrankungen und Cor Pulmonale. Schattauer Verlag, Stuttgart, New York 1975, pp. 172–174.
5. Diener, C. F., Burrows, B.: Further observations on the course and prognosis of chronic obstructive lung disease. Amer. Rev. resp. Dis. **111,** 719–724 (1975).
6. Howard, P.: Evolution of the ventilatory capacity in chronic bronchitis. Brit. Med. J. **3,** 392–395 (1967).
7. Johnston, R. N., McNeill, R. S., Smith, D. H., Legge, J. S., Fletcher, F.: Chronic bronchitis: measurements and observations over 10 years. Thorax **31,** 25–29 (1976).
8. Martin, C. J., Pardee, M., Dominik, J.: The diffuse obstructive pulmonary syndrome. Amer. Rev. resp. Dis. **93,** 383–389 (1966).
9. Sharp, J. T., Paul, O., McKean, H., Best, W. R.: A longitudinal study of bronchitis symptoms and spirometry in a middle aged mile industrial populations. Amer. Rev. resp. Dis. **108,** 1066–1077 (1973).
10. Vargha, G., Kováts, J.: Pulmonary Function Tests and Their Clinical Application. Akadémiai Kiadó, Budapest 1968, pp. 265–288.
11. Vargha, G.: Osztályos betegeink 10 éves légzésfunkciós kontrollvizsgálata. Tuberk. és Tüdőbetegs. **24,** 37–39 (1971).
12. Vargha, G., Rodics, K.: 15 year follow-up study of the lung function of patients after pulmonary damage. Bull. Physiopath. Resp. **11,** 199–200 (1975).
13. Vargha, G.: Gyógyult — dohányzó és nemdohányzó — tüdőbetegek légzésfunkciójának követése 15 éven át. Orv. Hetil. **120,** 379–381 (1979).
14. Vargha, G.: Újabb adatok az obstruktiv ventillációzavar kialakulásának és visszafejlődésének kérdéséhez. Pneumol. Hung. **32,** 76–78 (1979).

Book reviews

ROSE G. A., BLACKBURN H., GILLUM R. F., PRINEAS R. J.:

Cardiovascular Survey Methods WHO, Geneva,

178 pages, 10 figures 11 Annexes. Price: Sw. fr. 22.—

This second edition of the book is the work of four authors. What has made this new edition timely? Since the first edition in 1968 the use of preventive and epidemiological methods has increased and several new epidemiological methods have been elaborated.

The volume is divided into two parts; the first contains the general basic principles and the second the techniques of scientific investigation. Under the basic principles we find the aims of population studies (diagnosis of quantitative occurrences, distribution and nature of diseases) as well as the types of measuring (cross sectional and prospective control and experimental examinations). This chapter contains the advantages of certain examination procedures but also their limits. The second chapter introduces to bases of measurement. After emphasizing the necessity of standardization it demonstrates the significance of sensitivity and specificity that ensures the validity of applied tests. The third chapter gives an account of planning and representation. Population studies concerning the choice of samples can be done at random, confined geographically, and may originate from environmental or industrial groups. The authors give instructions by the help of mathematical formulas as to the size of retaining samples necessary for determination of the quantitative variables.

The three chapters of the second part give information on the contructions of survey questionnaires for epidemiological measurement, on carrying our physical examinations and on making special observations. Dealing with the methods of cardiovascular epidemiological measurements, the questions of research are foccused on smoking and dietary habits, physical activity, body weight and psychological characteristics. In case of prospective studies the book reviews the questions focussed upon the registration of symptoms. The physical examination should enter into the details of standardized methods of cardiac auscultation, the determination of the symptoms of heart failure, the conditions and correct procedure of blood pressure recording.

In the last chapter the authors survery the Minnesota Code for testing electrocardiograms, the ways of determining the heart size and its indexes (cardiac volume, frontal cardiac area, cardio-thoracic ratio), the parameters of ventilatory function and the laboratory tests.

The annex contains an ECG classification to be used in prospective studies, questionnaires and printed forms containing examination data. The points of measurement are illustrated by well-designed figures, the calculations are summed up in formulas.

Summing up, a sound knowledge of the contets of the book is of fundamental use in carrying out cardiovascular surveys by applying the most modern methods.

VERONIKA MORVAI

IPCS International Programme on Chemical Safety Environmental Health Criteria 17,
WHO, Geneva. 1981

Manganese

Manganese is an essential trace element for both man and animals. It is necessary for the formation of connective tissue, bone and for growth in general, and also for carbohydrate metabolism, the embryonic development of the inner ear and for reproductive functions. Some specific biochemical functions of manganese have been discovered such as the catalysis of the glucosamine-serine linkage in the synthesis of mucopolysaccharide in the cartilage. Food is the major of manganese in man and animals. The daily intake ranges from 2 to 9 mg depending on the consumption of foods like cereals and tea while have a high manganese content. The respiratory and gastrointestinal tracts constitute the major routes of absorption of manganese.

The toxic effects of manganese on the central nervous system were studied mainly in animals including the rat and the monkey. Manganese dioxide or dichloride aerosol inhalation, at concentrations 0.6–3.0 mg/m^3 daily for 1 hour over 4 months induced typical central nervous damages in monkeys. Chronic manganese poisoning is a hazard in manganese mining and processing, manganese alloy and dry-cell battery industriers. Post mortem findings in chronic manganese poisoning have shown lesions in the central nervous system. The lesions were especially severe in the striatum and pallidum and also in the substantia nigra. These findings combined with animal data and the effectiveness of 3-hydroxy L-tyrosine treatment implicate the dopaminergic pathway in the aetiology of the extrapyramidal manifestations of manganese poisoning. Early diagnosis is difficult in the absence of reliable indicators of exposure. Measurement of the manganese level in blood, urine and faeces serves as a useful guide to exposure. In an epidemiological study, the incidence of absorption and stillbirth was higher in wives of workers exposed to manganese for 10–20 years than in the control group. Toxicologically, the manganese fraction is of little importance, whereas the organic portion is part of a problem concerning this type of fungicide. The manganese tricarbonyl compounds are used as additives in unleaded petrol. In the future many other uses seem likely .There is little information concerning the mutagenicity of manganese. Processes as genetic recombination might be affected by manganese through its influence on enzymes that control the structure and metabolism of DNA. Manganese can be substituted by magnesium in the binding of two rebosomal subunits. Permanganate given to rats in 10 mg/kg doses daily produced an increase in the mitotic activity of bone-marrow cells. Manganous chloride has been reported to be mutagenic in the Ames-test. Few experimental studies have been conducted on the carcinogenicity of manganese and its compounds. When manganous sulfate was administered intraperitoneally to mice in doses of 10 mg/kg it produced a significant increase in the incidence of lung tumours. The book concludes that the data available were too meagre to allow an evaluation of the carcinogenicity of manganese in man and animals. In view of the data concerning the possibility of an increased use of organomanganese compounds as petrol additives in the future, it is recommended that epidemiological surveys should be conducted in communities exposed to annual mean concentrations of manganese in air exceeding 1 μg/m^3.

Environmental Health Criteria 18
Arsenic

This document contains some comprehensive reviews on the health effects of inorganic and organic arsenic compounds which are ubiquitous elements with metalloid properties in our environment. In nature arsenic is widely distributed mainly as arsenite or arsenate. In water,

usually arsenic is found and the methyated arsenic compounds have strong biological effects. Arsenic (III) oxide is produced as a byproduct in the melting of copper and lead ores and arsenic containing pesticides. A mean blood arsenic level of 0.00188 mg/kg (range, 0.0005–0.0038 mg/kg) was found among children living in country towns.

The acute effect caused by inorganic arsenic compounds is a gastrointestinal damage resulting in severe vomiting and diarrhoea. Further acute symptoms are muscular cramps, facial oedema and cardiac disturbances; shock may also develop as a result of dehydration. The fatal dose for man of ingested arsenic (III) oxide has been reported to range between 70 and 180 mg. The subacute effects involve mainly the respiratory, gastrointestinal, cardiovascular, nervous and haematopoietic systems. In reviewing available data in 1973, as well as in 1980, the IARC concludes that there was sufficient evidence to associate exposure to inorganic arsenic in man cancer of the lung and skin.

Environmental Health Criteria 19
Hydrogen sulfide

The concentrations of hydrogen sulfide in urban areas may be as high as 0.05 mg/m^3 (0.033 ppm). It is believed that workers are not usually exposed to hydrogen sulfide concentrations above the occupational exposure limit of 10–15 mg/m^3 (7–10 ppm). Massive exposures to hydrogen sulfide usually resulted from leaks in industrial gas streams containing high levels of hydrogen sulfide or from the slow, insidious accumulation of the gas of biogenic origin. In experimental animals, the effect of high doses of hydrogen sulfide and of cyanide are similar. They inhibit the enzyme cytochrome oxidase, interfering thereby with the consumption of oxygen by the tissues to the point where metabolic demands cannot be met. There is little information on the effetcs on experimental animals of long-term low-level exposure to hydrogen sulfide. Because it is both an irritant and asphyxiant gas the toxic effects are keratoconjuctivitis (gas eye), hyperpnoea, apnoea, pulmonary oedema, paralysis of the respiratory centres. Death is due to asphyxia unless spontaneous respiration is reestablished or artificial respiration is promptly provided. Chronic intoxication is a largely subjective state characterized by fatigue and believed by some to be a consequence of intermittent exposure to hydrogen sulfide at concentrations of 75–150 mg/m^3. Exposure to hydrogen sulfide in high concentrations occurs among workers in the oil, gas and petrochemical industries. Hydrogen sulfide in ambient air in concentrations of the order of the odour threshold has not been shown to have any significant biological activity in man or animals. Studies should be conducted in experimental animals on the cumulative neural effects of repeated and/or continuous long-term hydrogen sulfide exposure at concentrations that induce subacute or chronic intoxication.

ANNA TOMPA

IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans Vol. 27: Some Aromatic Amines, Anthraquinones and Nitroso Compounds, and Inorganic Fluorides Used in Dringking-water and Dental Preparations

IARC, Lyon 1982. 341 pages Price Sw. fr. 40.—

The previous volumes of the IARC monographs evaluating the carcinogenicity of different chemicals include some aromatic amines and anthraquinones which were used mainly as dyestuff intermediates. The present issue summarizes the specific information on the absorption,

distribution and metabolism of many aromatic amines. These data suggest that absorption in humans may occur via the respiratory and gastrointestinal tracts and through the skin. Aromatic amines and amides are readily metabolized by oxidation of their carbon or nitrogen atom in both man and animals. The formation of maleic acid and mutagenic carcinogenic metabolites appear to involve N-oxidation. More than 700 dyes, as listed in the Colour Index, can be made from aniline; few dyes based on aniline or aniline derivatives are, however, produced in commercially significant quantities. Aniline has been reported to occur in surface samples taken from some rivers in Europe and this was attributed to industrial chemical waste. Aniline was found to be present in several fruits, vegetables and in black tea. It has also been detected in tobacco smoke. Aniline hydrochloride tested in mice by oral administration produced no carcinogenic effect, but the same treatment in rats caused various malignancies of the mesenchymal tissue. In humans a high risk of bladder cancer has been observed in workers of the aniline dye industry. This, however, was probably due to exposure to chemicals other than aniline, because the evidence is limited for the carcinogenicity of aniline hydrochloride in humans and animals. Ortho-para-anisidine and its hydrochlorides are intermediated in the manufacture of synthetic dyes. There is sufficient evidence of the carcinogenicity of ortho-anisidine hydrochloride in experimental animals. The lack of human data makes it impossible reliably to evaluate its human carcinogenicity. Ten more chemicals of the aromatic amines are listed in this chapter. Half of them were characterized as carcinogenic to experimental animals. For the other five there is only a limited evidence of carcinogenicity.

In this book another group of chemicals, the anthraquinones, used as dye intermediates, is also discussed. In experimental animals there is sufficient evidence of the carcinogenicity only for 2-methy-1-nitroanthraquinone. In this respect no human data are available.

Two nitroso-compounds are evaluated in this monograph. N-nitrosodiphenylamine used in the rubber processing manufacture, was mutagenic in the Ames-test and caused bladder cancer in experimental animals. These data, however, mean only a limited evidence for its carcinogenicity. The other nitroso compound, para-nitrosophenylamine, is used as a chemical intermediate for dyes and as polymerization inhibitor during the manufacture of vinyl monomers. The data available are insufficient for an evaluation of its carcinogenicity in experimental animals. In the case of both nitroso compounds no data on humans were available, therefore no evaluation of their carcinogenicity in humans was possible.

The last group of chemicals evaluated in this monograph are the inorganic fluorides. Studies of water fluoridation and cancer are only reviewed in the book. None of the studies provided any evidence that an increased level of fluoride in water was associated with an increase in cancer mortality. The data available are inadequate for an evaluation of the carcinogenicity of sodium fluoride, the only inorganic fluoride tested in experimental animals. These critical analyses of experimental data are intended to assist national and international authorities in formulating decisions concerning preventive measures.

ANNA TOMPA

IARC Monographs on Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 28: The Rubber Industry.

IARC, Lyon, 486 pages. Price: 1982. Sw. fr. 70.—

This recent issue of the IARC Monographs discusses the probability that exposure to a chemical agent or its complex mixtures in a particular occupation will lead to cancer in man. All the pertaining data are summarized in the monograph. The choice of the field was given on

the basis of two main criteria i.e. that there are data connecting exposure and cancer in man, and there are evidences of human exposure.

The criteria of evaluation and evidence of carcinogenecity in humans has been emphasized in the previous IARC monographs. This issue is concerned with the tyre manufacturing and repair sector, cable-making and the manufacture of other rubber goods (automotive, mechanical engineering, building, footwear, furniture, textile, electrical, sports, etc.) The consumption level of natural and synthetic rubber is the highest in the USA and East Europe. The total world consumption of natural rubber was 3.85 million tons in 1980. There are many difficulties in determining the relation between exposure and disease in the rubber industry. When in 1949, 2-naphtylamine was shown to be a significant impurity in some of the rubber antioxidants, the direct causal link between exposure to the agent and the occurrence of bladder cancer was established with reasonable certainty. Now it appears that the removal of some suspect chemical compounds from the industrial process did effectively eliminate this particular occupational bladder cancer hazard, but mortality from lung and stomach cancer in the same groups of workers have been reported in both American and British rubber industries.

The book appears to be useful for the systematic monitoring of the industrial environment; these informations help to define the acceptable hygienic standards and codes of practice in a greater dissemination of safety regulations in prophylactic cancer research. The monograph gives detailed information about the history of the rubber industry. The reader will have a view of the manufacturing and the chemistry of rubber compounding. It describes the ways of exposure at the workplace and the environmental monitoring techniques. In the part on the biological effects it deals with certain epidemiological studies of carcinogenicity in humans.

In the Appendixes 1 to 3, the book gives the list of all chemicals and structural formulas occurring in the rubber industry. The detailed bibiliography and the cross index of chemicals makes the book particularly useful for industrial hygienists or researchers.

ANNA TOMPA

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Volume 33 Number 4

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